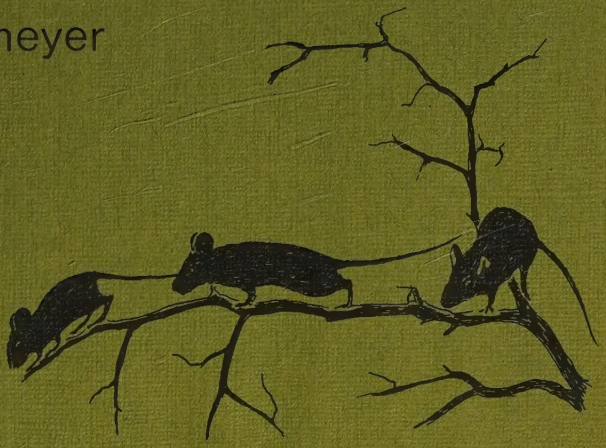
Interspecific behavioural niche separation in wood mice (Apodemus flavicollis and A. sylvaticus) and scent marking relative to social dominance in bank voles (Clethrionomys glareolus).

Inge Hoffmeyer



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II.Clethrionomys. Individual differences in scent marking, i.e.urine marking frequency and pattern, behavioural and chemical properties of the marking components and reaction to marking components were related to difference in male social rank, determined by male contest. Dominant males marked more frequently and the pattern was characterized by many trace- and small spot-urinations. Subordinates rarely trace marked.A positive correlation was also found between marking frequency and mating success of males.Estrogen-treated females were more attracted by dominant males, and by odors from these males. A marking component, hexadecyl acetate, was isolated by GC/MS in male markings and preputial gland secretion but not in metabolic urine. Dominant and subordinate males responded differently to this compound,according to rank,whereas females showed no clear reaction.

Key words Apodemus, behaviour, habitat, food, interaction, social behaviour. Clethrionomys, scent marking, social status, mate competition,olfactory responses.

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II. Clethrionomys. Individual differences in scent marking, i.e. urine marking frequency and pattern, behavioral and chemical properties of the marking components and reaction to marking components were related to difference in male social rank, determined by male contest. Dominant males marked more frequently and the pattern was characterized by many trace- and small spot-urinations. Subordinates rarely trace marked. A positive correlation was also found between marking frequency and mating success of males. Estrogen-treated females were more attracted by dominant males and by odors from these males. A marking component, hexadecyl acetate, was isolated by GC/MS in male marking urine and preputial gland secretion but not in metabolic urine. Dominant and subordinate males responded differently to this compound according to rank, whereas females showed no clear reaction.

UNIV. LEIDEN BIBL.

LIST OF PAPERS

This thesis is mainly based on the following papers, which will be referred to by their Roman numerals:

I. Hoffmeyer, I. Interaction and habitat selection in the mice Apodemus flavicollis and A.sylvaticus. - Oikos 24: 108-116 (1973).

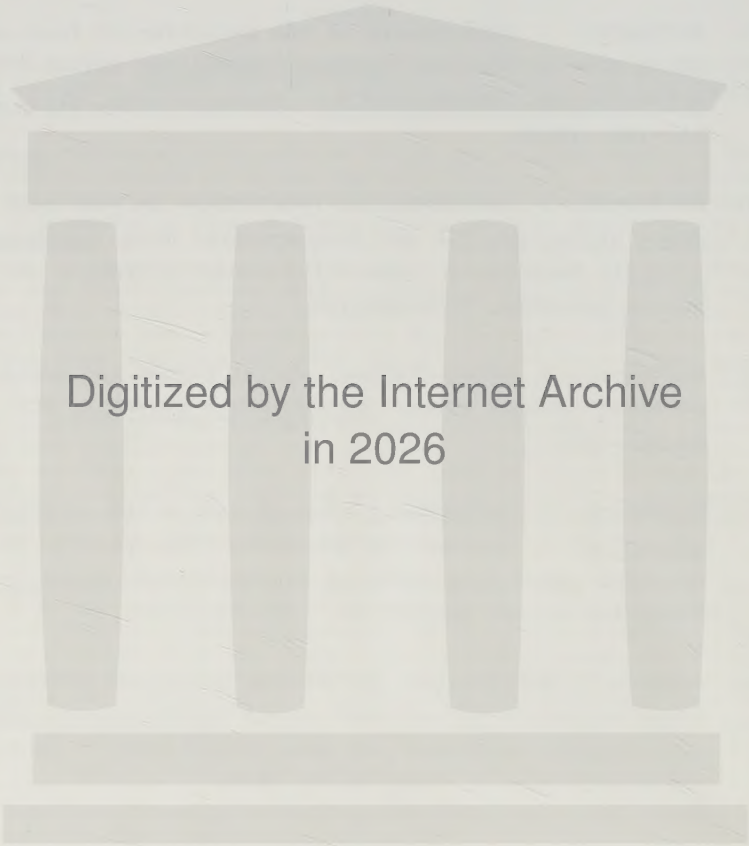
II. Hoffmeyer, I. Experiments on the selection of food and foraging site by the mice Apodemus sylvaticus (Linné 1758) and A.flavicollis (Melchior 1834).- Säugetierkdle. Mitt. 24: 112-124 (1976).

III. Hoffmeyer, I. Intraspecific interaction in wood mice (Apodemus sylvaticus L.) and yellow-necked mice (Apodemus flavicollis Melch.). A comparative study on spacing and cohesive behavior. - Manuscript.

IV. Hoffmeyer, I. Scent marking, social status and mating competition in bank voles (Clethrionomys glareolus L.). - Manuscript.

V. Hoffmeyer, I. Responses of female bank voles (Clethrionomys glareolus) to dominant vs subordinate conspecific males and to urine odors from dominant vs subordinate males. - Behavioral and Neural Biology 36: 178-188 (1982).

VI. Brinck, C. & Hoffmeyer, I. Marking urine and preputial gland secretion of male bank voles (Clethrionomys glareolus L.). Chemical analyses and behavioral tests. - (Accepted for publication J. Chem. Ecol. 1983).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SYNOPSIS

The thesis comprises studies within two different fields:

A) An analysis of behavioral differences between two closely related coexisting species of mice, Apodemus sylvaticus and A.flavicollis. The aim was to elucidate factors, which allow their coexistence (I, II and III).

B) An analysis of scent marking in relation to social dominance and with reference to possible functions in mate competition. Here the bank vole (Clethrionomys glareolus) was used as experimental animal (IV, V and VI).

In both cases differences resulting from, or enhanced by, competition were examined - in Apodemus interspecific, in Clethrionomys intraspecific (male - male) competition. The first case was mainly related to ecological factors, the second one to social factors (social dominance). To connect the two studies, I have in the Apodemus part (here) emphasized the discussion on intraspecific social behavior.

COMPARATIVE STUDIES ON APODEMUS SYLVATICUS AND A.FLAVICOLLIS

These are two closely related species of mice that long have interested European zoologists (see review by Jüdes 1982). Morphologically they are reported to be more similar in the south-eastern part of their common geographical range, than in the north-western part (Ursin 1956, Niethammer 1969). In my study area in Sweden they are easily distinguished (Fig. 1).

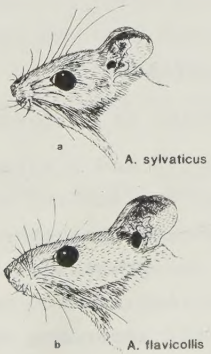

Fig. 1

The ecological differences between them are less clear. A.flavicollis occurs rather exclusively in woodland, whereas A.sylvaticus occurs in both woodland and grassland. Both are primarily granivorous. There seemed to be extensive overlap in both habitat and food requirements. Because of the overlap in utilization of space and food they are potentially competing species so segregation mechanisms allowing coexistence were expected.

Coexisting small rodent species are often separated by preference for different microhabitats (Rosenzweig 1977, Randall 1978, Dienske 1979). In granivorous rodents habitat selection appears to be the general basis of niche separation (Rosenzweig 1977), whereas a wide range of food items may be shared (Hansson 1971). Different species often have densities correlated with different structural features of the vegetation. The vegetation type apparently provides cues, which inform the individuals about habitat acceptability (Harris 1958, Wecker 1963).

The aim of my investigations was to study differences in behavior indicating niche separation.

The following questions were posed:

1. Do behavioral differences exist and if so can they be related to the use of different (micro)habitats? (I + Appendix);
2. Are there differences in food habits and foraging behavior? (II);
3. Are there differences in social behavior (social tolerance) which may reflect differences in social (spatial) organization? (III);
4. Which species is generally dominant at interspecific encounters? (I); and which are the consequences in terms of distribution?

RESULTS

Behaviors related to occupation of different habitats (I)

Newly trapped Apodemus flavicollis and A.sylvaticus were given a choice between a beech-wood and a grassland habitat in enclosures. A.flavicollis occurred about as much in both habitats, whereas A.sylvaticus occurred more in the grassland. The same pattern was found in 3 types of enclosures ranging from small indoor to large outdoor pens and with 3 different recording methods (I).

This general difference between the two species in distribution in the woodland-grassland enclosures could be related to behavioral differences. Adult A.flavicollis showed a stronger tendency to ascend the trees than A.sylvaticus. A.flavicollis also spent less time under cover on the ground, and ground cover was much more abundant in the grassland habitat. A.flavicollis moved about more freely than A.sylvaticus in areas without ground cover (e.g. in woodland), and latencies to leave a shelter on the ground were short compared with A.sylvaticus (I + Appendix).

Different propensities to enter open areas in the experiments could be related to the use of different types of vegetation cover. To habitat with ground cover (A.sylvaticus) vs habitat without or with little ground cover (A.flavicollis). The difference in behavior between the two species might also be the consequence of different susceptibility to predation. Adult A. flavicollis appeared to have a greater variety of antipredator adaptations (p.35), which allows this species to use habitats with poor shelter on the ground, and to behave less cautiously.

Different use of areas covered in different ways agree with reported observations in the field. A.sylvaticus was more numerous in ground covered areas, whereas A.flavicollis prevailed in areas of dense canopy, where the ground per se was poorly covered (Montgomery 1980a). Abramsky (1981) found a positive correlation between the density of A.sylvaticus and the percent of ground cover. Apparently, in contradiction to my findings on climbing, Montgomery (1980c) recorded no difference in the use of arboreal runways between the two Apodemus species. In my studies, the tendency to climb was clearly much stronger in A.flavicollis. Large tail to body length ratio has been used as an index of the climbing ability of Apodemus species (cf. Abramsky op.cit.), and A.flavicollis has a larger ratio than A.sylvaticus. Possibly lower arboreal runways are used similarly by the two species, while the difference in climbing tendency and skills becomes more marked at greater heights of larger trees.

Differences in food habits and foraging behavior (II)

A.sylvaticus fed more in grassland and A.flavicollis more in woodland, when identical food (pellets) was provided in both places. Thus habitat segregation occurred independently of food availability. Both species are primarily granivorous. Selection be-

tween beech (Fagus sylvatica)- and grass (Dactylus)-seeds, still on their plants, was investigated. Both seed types occur in the common habitat. A.sylvaticus took more of the grass spikes and also exploited them better than A.flavicollis. A.flavicollis on the other hand took more beech mast than A.sylvaticus and was more efficient in penetrating the spathes. The results indicate differences in "handling" and "travelling" times between the two species while foraging. There was also indication that A.sylvaticus more readily than A.flavicollis e.g. may switch from familiar to unfamiliar food, or (?) from small (grass) to large (tree) seeds. Preference by laboratory born animals was influenced by the food used during breeding.

I concluded that food is not the cause of habitat segregation. Habitat segregation causes different food habits, which are enhanced because of adaptations (innate predispositions) to utilize different food types.

Differences in social behavior (III)

In encounters between adult conspecific males trapped during the breeding season (May-August), A.sylvaticus generally showed more spacing behavior (attacks, chases, flights) than A.flavicollis which in general was more mutually tolerant and showed more contact-promoting behaviors. This observation was made in staged encounters between residents and strange intruders as well as within groups of 4 animals/group (III).

The results confirmed earlier observations during my habitat enclosure studies (I). They are related to ecological differences (see III-Discussion and p.10 - 12).

Difference in social behavior was also found when studying the ultrasonic behavior in the two species (Hoffmeyer & Sales 1977). A.flavicollis produced calls mainly in association with social encounters, while in A.sylvaticus the calls were often emitted by an animal moving about alone. These differences were related to and may serve to enhance the above mentioned major differences in non-ultrasonic behaviors. In A.sylvaticus sounds are probably used to space out the activities of individuals in time and space. In A.flavicollis on the other hand, the frequent ultrasound emissions during social contact behavior indicates that ultrasounds are used in very close-range communication.

The differences in the ultrasonic behavior may also reflect differences in habitat use of the kind suggested by my other results. Although the ultrasounds emitted by both species of mice are probably not audible to owls (Payne 1971), they are within the hearing range of cats (Evans 1968) and possibly of other carnivores. If, as concluded above, A.sylvaticus tends to live in more densely vegetated areas, it can afford to emit sounds often as they are attenuated or scattered more rapidly by the vegetation. A.flavicollis on the other hand, preferably using more "open" vegetation, should be more cautious as sounds would carry a greater distance. This could explain why A.flavicollis seldom emitted sounds when moving about alone, and mainly used the sounds in close-range communication.

CONCLUSION (I, II and III)

A number of behavioral differences could be related to different habitats: 1) Differences in individual behaviors, i.e. in cover utilization and in the tendency to (ascend) climb trees (I) including differences in anti-predator adaptations (p.35), and in foraging techniques (II). 2) Differences in social behavior reflecting differences in spatial organization could also be related to the differences in habitat, implying differences in predation and food (III). The results on social behavior thereby supplemented those on individual behavior in the conclusion that niche separation of the two species is mainly based on adaptations to different habitats.

I suggest that the eco-behavioral investigation on Apodemus helps elucidate the functional significance of body size differences in the coexistence of seed-eating rodents claimed by Bowers & Brown (1982) in connection with their study on relationship between local coexistence and body size ratios in desert rodents.

INTERSPECIFIC INTERACTION

Also interactions between the two species plays some role for space partitioning. In interspecific encounters, A.sylvaticus generally avoided A.flavicollis, and became inactive when A.flavicollis were active (I). Behavior involved in interspecific in-

teraction reflected the behavior employed at the intraspecific level. A.sylvaticus which intraspecifically showed more spacing behavior (III) generally fled at encounters with A.flavicollis (I) (which is the normal behavior of A.sylvaticus toward conspecific dominant strangers). A.flavicollis generally showed little overt aggression in intraspecific encounters (III) and behaved similarly in encounters with A.sylvaticus (I). In A. sylvaticus habitat displacement was observed, when A.flavicollis was present, both in terms of vertical (I) and horizontal restrictions (Hoffmeyer & Hansson 1974). In both cases the segregation due to habitat selection was enhanced: A.sylvaticus was restricted more completely to the ground level (I) and to grassland (Hoffmeyer & Hansson op.cit.).

PREDICTIONS ABOUT SOCIAL ORGANIZATION

Comparative studies on closely related species is a useful method for understanding how social organization might be influenced by ecological pressures. One of the principal findings of ethologists Crook (1965), Eisenberg (1967), Jarman (1974) and Crook et al. (1976) is that social organization of animals can be related to a limited number of environmental variables, such as habitat, predation and food.

By comparison with general correlations found between social organization and these ecological parameters in other species, the difference as to predominance of distance-promoting behavior in A.sylvaticus vs predominance of contact-promoting behavior in A.flavicollis could be related to major ecological dichotomies (III-Discussion).

Gross habitat difference - forest vs open field - appears to cause differences in the individual behavior of small rodents (Fig. 2, p.33). The same is likely to be the case with social behavior. The more stable conditions of (mature) woodland favor more stable societies, and climbing habits may imply a general relax on violent agonistic interactions.

Micro-habitat characters, i.e. structural features/vegetation types, provide different anti-predator protection. The vegetation characters also give different physical limitations to group cohesion. Concerning predation avoidance two basic strategies can be distinguished: "Concealment" (A.sylvaticus), which should lead

to spacing of individuals; or "early detection and escape" (A. flavicollis) which often leads to group living. Concerning influence on group cohesion the density of vegetation is important. In denser vegetation individuals may have more difficulty in keeping together. Groups of antelopes are generally smaller in forest than in open land habitats (Jarman op.cit.). However, from a small rodent "view" it may be just opposite. Here a grassy habitat of open fields represents the denser vegetation type, in contrast to often "open" areas on a forest floor.

Concerning food, not only the distribution in space and in time is important; but also the handling and "mode of assembly" (Eisenberg op.cit.). Differences in climbing and hoarding determine differences in food availability. Through better climbing skills, A.flavicollis may e.g. exploit the beech seeds before A.sylvaticus. By the use of food types that can be hoarded, the availability period of these foods is prolonged.

In the prediction about social organization in Apodemus I focused on the difference in social tolerance (III), which reflects a tendency of spacing in A.sylvaticus, while in A.flavicollis individuals may live in closer contact. I tentatively suggest the following opposite trends:

For A.flavicollis the stability of the habitat, including refuges nesting places, hibernacula and sites where preferred food is produced (seed crops connected with big trees), should promote long-term site-attachment. On the other hand, the great variation in food abundance between years should promote variation in numbers of animals (group size). The social organization of A.flavicollis therefore is predicted to be long-term family--territories attached to some big trees. In years of low seed--production, the food provided by the trees (incl. buds, larvae etc.) would be sufficient to support a family unit of a few individuals having the potential for increase, when food gets abundant. Increase in A.flavicollis numbers appears to follow mast years (Hansson 1971, Hoffmeyer & Hansson 1974). The low ground vegetation density in beech forest provides better possibilities for animals keeping together when foraging on the ground. The advantage of group living as an anti-predator strategy (mutual vigilance) further favors sociality in the open forest floor habitat of Apodemus flavicollis.

Conversely, A.sylvaticus lives in a successional (and thus more unstable) habitat: Open grasslands, often in the form of

small temporary openings in the forest. The even distribution of food, less rich in energy content, and the rather dense cover of the grass favoring a concealment strategy against predators, are preconditions for a more spaced out and solitary social system, with shorter periods of parent-offspring association.

According to modern evolutionary theory sociality is favored by gene sharing, has evolved by kin selection (Hamilton 1963). From the difference in sociality between the two Apodemus species one would predict greater genetic relatedness between A.flavicollis individuals as well as a mechanism for kin recognition. Kin recognition could be facilitated by delayed dispersal of young; but recognition of unfamiliar kin seems possible too. Kin recognition that was not based on prior contact has been reported from a study on white-footed deer mice (Peromyscus leucopus) (Grau 1982).

Mating systems

Generally, the greater the potential for males to monopolize resources or mates, the greater the potential for polygyny (Emlen & Oring 1977). Therefore differences in mating systems may result from the a.m. differences in ecological conditions. The mating systems of the two species are almost unknown, but traping results (Montgomery 1979, Hoffmeyer unpubl.) suggested some difference.

In A.flavicollis the number of females aggregated within an area should vary according to the abundance of available tree seeds. One male may well be able to control all females within a limited area, but occurrence of many females might lead to a situation, where the dominant male is no longer able to inseminate them all (cf. Waltz 1981). Synchrony in estrous often occurs among female mice living in close vicinity of each other.

Consequently, the dominant A.flavicollis male may have to allow other males to mate with the "excess" females. If these males are close relatives (brothers, sons) the dominant would still benefit by increasing his "inclusive fitness" (Hamilton 1963). This, and the advantage of having associate males to help keeping competitors away from the site may explain a tolerant behavior of dominant A.flavicollis males (see III). The explanation of the behavior of the (subordinate) associate males probably is that their costs of leaving are too high. Being more

specialized in their ecological requirements, A.flavicollis males should run greater risks than A.sylvaticus males of not finding suitable habitats. Furthermore, the advantages of sharing or "inheriting" a site with superabundance of vital food, and mates, may keep young males from leaving in spite of not getting the maximal reproductive output. Other causes of A.flavicollis social tolerance have been mentioned (III). Taken together these evidences may explain, why several reproductively active male A.flavicollis often occur together within a limited area in nature, and often show little aggression toward each other.

In A.sylvaticus, the number of females within an area should in general be lower and more constant. The food is of poorer quality and more evenly distributed. This gives better possibilities for exclusive control by single dominant males. Other males should be chased or be socially suppressed if staying. This corresponds to the results of Flowerdew (1974) from the field.

One may expect some convergence in social systems of the two species when ecological conditions are most similar: The social organization - mating system of A.flavicollis should approach that of A.flavicollis, if A.sylvaticus lives in beech forest without, or with low numbers of A.flavicollis. However, the long term adaptation to different habitats no doubt has caused too profound changes (evolutionary constraints) in reproductive behavior and physiology for total similarity.

In summary, I suggest that besides differences in microhabitat and body size, the difference in social systems of the two Apodemus species described here also contributes to their possible coexistence.

The Apodemus investigation led to the studies on chemical communication in the bank vole (Clethrionomys glareolus). This small rodent which belongs to a different taxonomic group, the microtines or voles, lives in the same habitats as both Apodemus species, but mostly in forest. It is more easy to handle and keep in the laboratory than Apodemus.

Studies on chemical communication are of great interest for studies on small rodents. It is a major means of communication in these animals. Probably scent

signals used in agonistic interactions are understood between species like e.g. visual or auditory threat signals in birds. Furthermore, in areas where hybridization may occur, signals used in mate choice should diverge as a means of ethological isolation. The study of signal systems allowing coexistence of conspecific males was also suggested by the previous study (p.13). Consequently, chemical communication in relation to social dominance and mate choice became the main subjects.

Illustration of the principle of segregation of A.sylvaticus (Asy) and A.flavicollis (Afl) in relation to vegetation types. The difference in sociality is assumed to contribute. The arrows picture spatial and temporal separation of A.sylvaticus activities.

Fig. 2

SCENT MARKING IN THE BANK VOLE (CLETHRIONOMYS GLAREOLUS L.)

SOCIAL DOMINANCE AND MATE COMPETITION

Bank voles (or "forest voles" in Swedish) live mainly in or near forests. They climb, are relatively more granivorous than field voles (Microtus agrestis), but also eat fruits, leaves a.o. (Hansson 1971). Microtus agrestis is more confined to open field grassland habitats and is an extreme herbivore (Hansson op. cit.).

Site tenacity is reported to be generally strong during breeding in the female forest rodents of the genus Clethrionomys (review by Wiger 1982). While overt aggression is more frequent among Microtus agrestis males, Clethrionomys males may have stable dominance relations throughout the breeding season (Viitala 1977, Sørensen 1982 and in prep.). This may indicate a greater role of signals in Clethrionomys interactions (Viitala op.cit.).

Generally, communication by scent is well developed in species living in habitats where the possibility of visual and acoustic signals is limited. In mice, urinary cues are important both in male - male and in male - female interactions, and have a great influence of reproductive functions (review by Bronson 1979). Similar functions were suggested for the bank vole (Godfrey 1958, Johnson 1975, Christiansen 1980). The present experiments were made with animals from a South Swedish bank vole population. They were concerned with proximate functions, but the present part includes a discussion of ultimate functions (selective advantages) too.

My aim in the first study on the bank vole was to examine urine marking of males in relation to their social dominance and in relation to females (IV). Male dominance was defined by highest attack frequency (and correlated behaviors) shown toward strange males and subordinate group mates in intruder tests (see p.73).

The second study (V) was concerned with the effects of intermale dominance relations on female choice of mates.

In the third study substances deposited by male urinary marking were analysed chemically and the reactions of female and male bank voles to the marking compounds were tested (VI).

RESULTS

Male - male dominance relations were correlated with clear differences in marking frequency and pattern (IV), behavioral reactions to chemical components and chemical properties of marking substances (V,VI). Dominant males marked significantly more frequently than subordinate males, and a specific type of marking, "fine-trace"-urinations, were more numerous (IV). The marking frequency by subordinate males was significantly lower than that of the dominant males, both in the presence of a strange male intruder, and in the presence of females. Inhibited marking by subordinates was found even in the absence of the dominant male, when the subordinate was confronted with a strange male or female intruder in its own home cage. As the females responded postively to male marking substances and as the subordinate males showed no increase in marking frequency during short-term encounters with females, even when the dominant male was absent (IV), the difference in marking behavior between males is likely to be of some significance in their competition for mates. A positive correlation was indeed found between the marking frequency of individual males (of unknown social status) and the mating success of these males (IV). Female bank voles showed a preference for dominant males over subordinates (V), and they were more attracted by odors characteristic of dominant males (V and VI). However, it appeared that females were indifferent to a specific marking compound (hexadecyl acetate) to which the males reacted - subordinates by avoidance and dominants by approach (VI + Appendix). Chemical analyses had shown the presence of hexadecyl acetate in preputial secretion and marking urine but not in metabolic urine of males (VI).

DISCUSSION

Male - male competition in voles with special regard to the functions of marking

The following basic principles are important: 1) Male-male competition is strongest when the operational sex ratio is skewed toward males. Female voles generally live spaced out during the reproductive period (Wiger 1982) so successive polygyny is possible. 2) Male microtines must invest relatively much in mating because female voles are reflex ovulators (review by Westlin 1983) and thus prolonged mating activities are required to

assure fertilization and pregnancy. Consequently, a good strategy for a male vole should be to banish male competitors well in advance of mating. Whether competition is avoided mainly by spacing or social suppression depends on how females are spaced, and this again is determined by habitat and food parameters as previously discussed in the section on Apodemus.

Possible reasons for male coexistence were mentioned (p.13). A dominant male should preferably allow younger relatives to stay within his range, and I here tentatively suggest a further reason concerned with chemical communication: Dominant male bank voles might benefit from letting subordinates stay as these males could help keeping the dominant's scent markings active over time. In fact, subordinate males generally urinated on the same places as the familiar dominant, while they avoided urination on places of strange dominants (own observations). The urine of subordinates being by itself of little effect might by mere humidity renew the olfactory effects of dried substances earlier deposited by the dominant. Moisture clearly enhanced the voles' reactions to drying components; (own observations).

But why should a subordinate male stay and accept the high costs of suppression and reduced reproduction? In general he should do so when the costs of dispersal are high and there may be some advantage in sharing the range of dominants (review e.g. by Rohwer & Ewald 1981). Since site attachment of breeding females of Clethrionomys is stronger than in Microtus (Viitala 1977) there is an important advantage for subordinate male Clethrionomys to stay within the range of dominants as they thus have higher chances of taking over females. A high predation rate on the small rodents should increase the subordinates' chances. Coexistence of dominant and subordinate males is facilitated if they have a system of communication, which reduces overt interactions.

Is male marking behavior a segregation mechanism?

Coexistence between dominant and subordinate individuals may be facilitated by differentiation of signals, such as found in the difference in marking between dominant and subordinate male bank voles. It constitutes a sort of segregation by odor signals. Not only the quantity of markings differs, but their odor quality as well.

High marking frequency was correlated with high social status (IV) and dominant male bank voles reacted by approach to the marking compound hexadecyl acetate (VI + Appendix). Inhibited release of this marking compound by subordinate male bank voles might therefore be one way of signalling subordination (i.e. by sort of "olfactory concealment") in presence of dominants. This would facilitate coexistence. But also other signals of subordination are important in voles (auditory signals and submissive postures). The high marking frequency by dominants could act as an assurance against "cheating" by simple odor mimicry, as it involves higher costs (energy, exposure to predation) for the male and is normally "supported" by further kinds of dominant behavior. High frequency scent marking by a resident (dominant) male in an environment allows conspecific intruders to quickly assess the identity of the resident/dominant, and adjust their behavior accordingly, when they meet (Gosling 1982).

Subordinate males should be able to increase marking during encounters with females when the dominant is absent. This, however, did not occur in short-term encounter tests with the low ranking male bank voles (IV). The social inhibiton of marking in these adult males has shown that although androgen-dependent marking pheromone is produced it may not be externally voided. But, also the synthesis of marking compound may be affected by long--term subordination (Brinck, Hoffmeyer & Westlin, 1983). A delay in "relief from such suppressive effects of subordination" probably results from physiological constraints. In my experiments these effects may have been enhanced by the interactions between dominant and subordinates elicited by the "intruder method" (p.73) Gustafsson (1983) found no suppression of sexual maturation in presence of a familiar adult male, whereas confrontations with unknown animals caused a marked suppression.

Mate competition and sexual selection by scent

Different characteristics may be promoted by sexual selection dependent on their respective functions in male contest and female choice. If sexual selection occurs by the effects of male contest, then male characteristics which are advantageous in male--male interactions, should be favored during evolution. This means large body size/physical strength in animals showing spacing

mainly by overt aggression, and possibly also the development of mechanisms and structures needed for the production and emission of odor signals in those animals where agonistic scent communication is important.

Sexual selection by scent is a possibility in mammals (Darwin 1901 cf. Blaustein 1981). Special odors may be functionally equivalent to secondary sexual characteristics (bird plumage, deer antlers). Many small mammals are sexually dimorphic through odor (Stoddart 1974).

In the bank vole, sexual dimorphism in the size of the preputial glands (Christiansen et al. 1978), and in the pattern of urinary marking (Johnson 1975) may indicate that sexual selection by scent occurs in this species. There is no clear sexual dimorphism in body size.

In the present experiments male-male interactions resulted 1) in differences in urine marking frequency and pattern (IV), 2) in differences in urine odor quality (V and VI) and 3) in differences in the males' reactions to odors (VI). All the mentioned evidence gives a basis for intrasexual selection.

Also female choice may be important. Some evidence now exists, that female mice and voles are more attracted by cues from dominant males (Jones & Nowell for mice; Hoffmeyer for bank voles, Huck & Banks for the brown lemming). But the precise sources of the olfactory cues are still unclear. Other kinds of male cues are probably also needed for their choice of mate (ultrasounds, tactile stimuli). These cues may similarly vary with male rank (Nyby et al. 1976, Dewsbury 1981, Huck & Banks 1982). Furthermore, just as males may employ different strategies in getting access to females, females might differ as to the male qualities preferred.

In summary: According to the present results, both male contest and female choice might be involved in sexual selection by scent, but apparently not promoting the same olfactory characteristics. The effects of male contest could be to promote mechanisms involved in the production and external emission of the marking component hexadecyl acetate and in male reactivity to this component. The effects of female choice might be to promote the production of other male olfactory cues.

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When not more has come out of so much good will I really only have myself to blame!

OIKOS 24: 108–116. Copenhagen 1973

Interaction and habitat selection in the mice Apodemus flavicollis and A. sylvaticus

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Hoffmeyer, I. 1973. Interaction and habitat selection in the mice *Apodemus flavicollis* and *A. sylvaticus*. – Oikos 24: 108–116.

Males of *Apodemus flavicollis* (Melchior) and *A. sylvaticus* (L.) were given a choice between a grassland and a beech-wood habitat. Newly caught animals were tested alone or together in three types of enclosures ranging from small indoor to large outdoor pens. Distributions were recorded by direct observation, automatically by an event recorder, and by trapping. Interactions and other behavioural traits relevant to habitat selection were studied.
In all three types of enclosures, the relative distribution pattern of the two species was such that *A. flavicollis* occurred about as much in both habitats, whereas *A. sylvaticus* occurred more in the grassland. *A. flavicollis* was also more active in a novel environment and showed a stronger tendency to climb. In interspecific encounters this species clearly dominated *A. sylvaticus*, generally in a passive way. It was less aggressive in intraspecific encounters than *A. sylvaticus*. The presence of *A. flavicollis* inhibited activity of *A. sylvaticus* and increased its occurrence in the grassland. When tested together the two species tended to segregate vertically.

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Самцам *Apodemus flavicollis* (Melchior) и *A. sylvaticus* (L.) был предоставлен выбор между местообитаниями луговыми и на древесине бука. Только что пойманные животные были исследованы по-отдельности и вместе в 3 типах вольер, от маленьких – в помещении до больших загонов на открытом воздухе. Распределение животных регистрировалось прямыми наблюдениями, самописцами и методом отлавливания. Изучали взаимодействие других особенностей поведения, проявляющихся при выборе местообитания. Во всех 3-х типах вольер относительный характер распределения двух видов показал, что *A. flavicollis* встречается одинаково в обоих местообитаниях, а *A. sylvaticus* больше предпочитает луговые участки. *A. flavicollis* были также более активны сильную тенденцию к лазанию. В междувидовых столкновениях этот вид заметно подавлял *A. sylvaticus*, преимущественно пассивным путем. Он также был менее агрессивным, чем *A. sylvaticus*. Наличие *A. flavicollis* подавляло активность *A. sylvaticus* и повышало его численность в луговых местообитаниях. При совместном содаржании оба вида обнаруживали тенденцию к вертикальной сегрегации.

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1. Introduction

It is an axiom of ecological theory that different species of animals are able to select the kind of habitat which most suits their needs and that the choice of habitat by one species may be affected by the presence or absence of another with similar requirements. The process by which a habitat is selected is to a large extent elusive, but it seems that, at least in small mammals, the ability to select the correct habitat is partly inherited and partly learned by experience (Wecker 1963). My aim in this study was to analyse the kinds of choices made by two closely-related species of rodents when faced with two kinds of environment, woodland and grassland. The two species, *Apodemus flavicollis* (Melchior) and *A. sylvaticus* (L.) are known from observations in the wild and from trapping records to favour rather different habitats, *A. flavicollis* occurring almost exclusively in woodland, while *A. sylvaticus* occurs in both woodland and grassland. Thus, there is considerable overlap between them, the extent of which may vary geographically and seasonally.

To investigate the choices made by individuals of these two species the mice were presented with a grassland and a woodland habitat and with two artificial habitats presenting characteristics of grassland and woodland. In addition the behaviour of the two species was studied in an attempt to determine how habitat selection might operate in nature. The investigation was carried out at the Stensoffa Ecological Station in southern Sweden during May–November 1971.

2. Material and methods

2.1. General

All the mice used were captured in the vicinity of Stensoffa Station. Only males were tested. Before testing the mice were kept in captivity only for about 2–4 days and for 10 days at a maximum. They were housed in a room with the natural light-dark photoperiod and without heating, and were kept separately in plastic cages (40 × 25 × 30 cm) on wood shavings. Food (standard pellets) and water were provided without limit. Combinations of toe-clipping, colour marking, and fur-clipping were used for species and individual recognition. A few animals were used twice, but then in different experiments and never in the same test enclosure.

2.2. Experiments concerning habitat selection

Test enclosures of three different sizes were used, ranging from small (1.44 + 1.44 m^2) to quite big (150 m^2), all containing a grassland half with dense and sheltering ground vegetation and a beech-wood half without ground vegetation (Figs 1–3). Food and water were provided without limit in both habitats. In the indoor enclosures (Exps. I–II), the grassland habitat consisted of turves of grass, mainly *Dactylus glomerata* (L.), placed to form a continuous surface with a few open

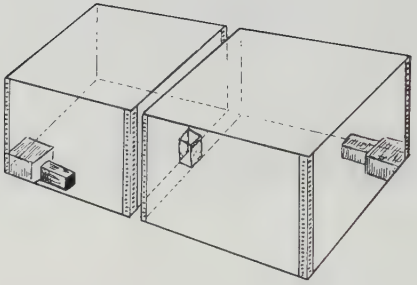

Fig. 1. Small indoor enclosures used for Exp. I. Size of each box: 1.2 × 1.2 × 0.8 m. Walls made of masonite. In one of the boxes was the "beech wood" and the other the "grassland". Each habitat contained a nest box (15 × 15 × 10 cm), a feeding dish with pellets covered by wire mesh to prevent hoarding, and a drinking tube. Between the boxes, and for each nest box, there is a wooden runway provided with a mercury switch attached under a bascule bridge and connected to an event recorder.

spots; the woodland habitat consisted of wood logs (ø = 6–8 cm) mounted vertically 0.5–1.5 m from each other, with fresh beech branches suspended on top, on a wooden frame provided with wire-mesh (mesh: 3 cm); the floor of the woodland was a layer of soil covered with dead beech-leaves and a few scattered branches and logs. The grass was watered between tests and some of the turves, dead leaves and beech branches renewed; nesting material was exchanged, and food-dishes and water tubes cleaned. In the outdoor en-

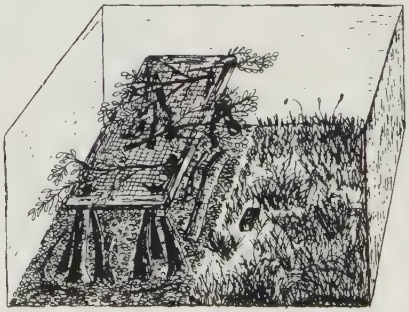

Fig. 2. Large indoor pen used in Exp. II. Size 4.0 × 4.0 × 2.0 m. Walls made of sheets of metal. The two habitat sections were separated by a 0.3 m wide boundary free of vegetation. In the grassland, open spots between the turves permitted the sight of mice moving about. In each habitat, two feeding dishes and water tubes were placed at the end wall in front of observation windows made of one-way glass. Ten traps scattered around in each section served as further feeding places.

Fig. 3. Large outdoor enclosure used for Exp. III. It was placed across a sharp boundary between a beech wood and a field. Size 30 × 5 m. Walls made of aluminium sheets (2.50 × 1.25 m) dug 0.40 m into the ground and supported by wooden poles. Each habitat was provided with 4 transversal rows of traps (3 traps per row) placed at equal distances from each other and from the walls of the enclosure. The traps were used as feeding dishes and provided with rolled oats *ad lib.*

closure (Exp. III) aluminium sheets were wrapped around the tree trunks about 1 m above the ground so as to prevent the mice from escaping.

In all these experiments the duration of each test was two nights and the day between them. The mice were released at the habitat boundary in the late afternoon and removed again on the second morning. One mouse was used per test in Exp. I, and 8 in Exps II–IV (4 of each species where 2 species were tested together).

The following account deals with some details concerning the individual experiments.

2.2.1. Experiment I

This experiment was made in the 1.44 + 1.44 m^2 double indoor enclosure (Fig. 1), in July–September. Two sets of these boxes were mounted parallel to each other and with alternating habitats to allow two tests to be made simultaneously. They were placed in a way that the grassland habitats and the woodland habitats received equal amounts of natural light from a window. Each morning the nesting site, and the food and water consumption of the test mice were registered. The time spent in each habitat was measured by mercury switches on the runway bridges connected to an event recorder.

2.2.2. Experiment II

This experiment was made in the 16 m^2 indoor enclosure (Fig. 2), in May–June. Observations were made from behind a screen placed at the top of one of the walls over the habitat boundary. White light was used, since pilot observations had shown that the mice were as active in white as in red light during the short night of this season. Observations were made during a total of 7 × 30-min periods per test: 4 on the first and 3 on the second night, between 2230 hr and 0130 hr. Within each 30-min observation period, the positions of all individuals visible were recorded at 5-min intervals. For each individual scores were summed for wood- and grassland habitat respectively, and "preference" was assigned to the habitat receiving the higher score.

2.2.3. Experiment III

This experiment was made in the 150 m^2 outdoor enclosure (Fig. 3), in September–October. The woodland habitat was somewhat altered between the two series of tests, (conditions I and II); the grassland habitat remained unchanged. In the first part of the experiment, the woodland habitat provided poor shelter and climbing possibilities (the latter due to the presence of the aluminium sheets wrapped around the tree-trunks); in the second part, the woodland was changed: 1) Better ground cover was provided by placing a heap of branches in the centre of the section and scattering more branches on the ground; 2) climbing possibilities were improved by supplying 8 vertical logs with small sitting platforms covered by branches.

Before each test, the 24 traps were baited with rolled oats and left open. The next day, the traps were rebaited and closed. On the following morning, the place of capture of each individual was registered. The capture was almost always 100 per cent of the test animals introduced.

2.2.4. Experiment IV

This experiment was made in September–October, in the 16 m^2 indoor pen, which this time was provided with artificial habitats: A "woodland" made of lattice work and a "grassland" made of hay arranged in tufts. Nest boxes were placed on the floor of both habitats, and on a horizontal wire-mesh "roof" on top of the

lattice work, 1 m above the ground. In the "woodland", 7 nests were placed on the "roof" and 6 on the floor; in the "grassland" 10 nests were placed on the floor, partly under and between the tufts of hay. Tests were made with 4, and with 8 individuals of each species per test. In the latter, the 8 *A. sylvaticus* were tested alone first, for the usual 2 nights and 1 day, and then 8 *A. flavicollis* were added. The mice were released between 1800–2000 hr (a time at which the mice were quite active at this season) and their behaviour was watched for an hour after introduction. In the morning, the nesting position of each individual was registered.

2.3. Registration of behavioural data

This part of the investigation was centered around three main points: interaction (interspecific and intraspecific), activity in a novel environment, and climbing tendency.

2.3.1. Interaction

During the observation periods of Exps II and IV each encounter between any two test mice was recorded and the individuals involved, site of the encounter, category of interaction, as well as outcome of the encounter were noted.

Two kinds of interaction were distinguished: 1) "Dominance" encounters. One of the individuals induced retreat of the other. 2) "Neutral" encounters. Neither individual induced retreat of the other; the mice were tolerant of each other, indifferent or amicable.

"Dominance" encounters were further divided into: "Active dominance", i.e. the retreat of one was induced by agonistic behaviour (threats, attack, chase) of the other, and "passive dominance", i.e. the retreat of one was induced by the simple, non-aggressive approach or proximity of the other.

2.3.2. Exploration and tendency to climb

In Exp. IV, all individuals exploring were noted at 5-min intervals after the mice had been introduced into the pen. Furthermore, the tendency for an individual's first attempt to climb to the top of the lattice work 1 m above the ground was recorded.

Tab. 1. Comparison of the choice of habitat of *A. flavicollis* and *A. sylvaticus* males tested individually in indoor enclosures of 2.88 m² (Exp. I). Each mouse was given the choice between a beech-wood habitat and a grassland habitat of 1.44 m² each. The nesting position was registered on the first and second mornings after introduction.

Section chosen on two consecutive days for nesting	No of individuals *A. flav.*	*A. sylv.*
W – W	9	6
G – G	8	20
W – G	6	1
G – W	2	0
Total no of indviduals tested	25	27

W – section with beech-wood habitat.
G – section with grassland habitat.

2.4. Statistical methods

In all comparisons of pairs of observed values, χ^2-tests are applied. Sometimes, two observed values are tested against a hypothesis of no difference (i.e. $p = q = 1/2$); here χ^2-tests are applied too, except when the sum of the two values is ≤ 25, in which case a Binomial-test is used. In the following, χ^2-values are given only where the two *Apodemus* species are compared.

3. Results

3.1. Habitat selection

3.1.1. Choice in small indoor enclosures

As shown in Tab. 1 (Exp. I), there appear to be two differences between the species. First, as regards individuals making the same nesting choice for two days, *A. flavicollis* showed no preference between the woodland and grassland whereas *A. sylvaticus* preferred the grassland section ($p < 0.01$). The difference between the two species is significant ($\chi^2 = 4.036$; $p < 0.05$). Secondly, as regards individuals changing their nesting site from the first to the second day, more *A. flavicollis* than *A. sylvaticus* changed their habitat during the two days of the experiment.

In addition, the time spent in each of the two habitats during the activity period of the second night was measured for 16 individuals of each species chosen at random from the total tested. Twelve of the 16 *A. sylvaticus* spent more time in the grassland, whereas 10 *A. flavicollis* spent more time in the woodland section, and 6 more time in the grassland section. The difference between the two species is significant ($\chi^2 = 4.571$; $p < 0.05$).

3.1.2. Relative use of each habitat in a large indoor pen

As shown in Tab. 2 (Exp. II), *A. flavicollis* showed no clear preference between the two habitats ($p = 0.067$ and $p = 0.291$, Bin.-test). *A. sylvaticus*, however, preferred the grassland habitat, and the difference is significant in both situations ($p < 0.001$ and $p \leq 0.01$, Bin.-test). It appears that a relatively higher number of *A. sylvaticus* occurred more frequently in the woodland habitat when *A. flavicollis* were absent than when they were present. The difference between the two species in relative habitat use, however, is significant in both situations. ($\chi^2 = 21.989$; $p < 0.001$, and $\chi^2 = 6.689$; $p = 0.01$).

3.1.3. Occurrence in a big outdoor enclosure

As shown in Tab. 3 (Exp. III), with poor hiding and climbing facilities in the beech-wood section, *A. flavi-*

Tab. 2. Comparison of the occurrence of *A. flavicollis* and *A. sylvaticus* males in the beech-wood and grassland habitats of a 16 m² indoor enclosure (Exp. II). The figures represent the totals of individuals having received the higher score for the respective habitat (cf. Sect. 2.2.2.). The relative habitat use of the two species is compared for the situations "both species together" (4 + 4 per test) and "either species alone" (8 per test).

Type of experiment	*A. flav.* No. of individuals observed most often in:		*A. sylv.* No. of individuals observed most often in:	
	W	G	W	G
A) Two species together in the enclosure	15	7	0	21
B) Either species alone in the enclosure				
A. flav. alone	8	5	–	–
A. sylv. alone	–	–	3	15

W – section with beech-wood habitat.
G – section with grassland habitat.

Tab. 3. Comparison of the occurrence of *A. flavicollis* and *A. sylvaticus* males in the beech-wood and grassland habitats of a 150 m² outdoor enclosure (Exp. III). The two species were together in the enclosure (4 + 4 per test). Results are based on trapping during the second night after introduction of the mice. The woodland habitat was changed between the two series of tests of this experiment (cf. Sect. 2.2.3.).

Type of experiment (the two species tested together)	*A. flav.* No. of individuals trapped in		*A. sylv.* No. of individuals trapped in	
	W	G	W	G
I) with poor hiding and climbing possibilities in the woodland section	3	16	2	18
II) with improved hiding and climbing possibilities in the woodland section	13	11	6	22

W – beech-wood half of enclosure.
G – grassland half of enclosure.

collis as well as *A. sylvaticus* were trapped in much greater numbers in the grassland than in the woodland habitat. The difference is significant for both species ($p = 0.004$ for *A. flavicollis*, and $p = 0.002$ for *A. sylvaticus*, Bin.-test). There is no significant difference between the two species. When hiding and climbing facilities were improved, the distribution of *A. flavicollis* changed, whereas that of *A. sylvaticus* was about the same. This time, *A. flavicollis* were trapped in almost equal numbers in the two habitats, but *A. sylvaticus* were again caught more frequently in the grassland habitat ($p < 0.01$). Thus, when the two species were together having the choice between a beech-wood habitat with a certain amount of climbing and hiding facilities, and a grassland habitat, *A. flavicollis* occurred evenly in both habitats, whereas *A. sylvaticus* kept to the grassland; for the difference between the two species in situation II, $\chi^2 = 5.973$; $p < 0.05$.

A. flavicollis showed a clear difference in occurrence depending on conditions in the woodland habitat – between situation I and situation II – ($p < 0.01$). *A. sylvaticus*, on the other hand, showed no difference ($p < 0.25$).

Apparently, *A. flavicollis* influenced the distribution of *A. sylvaticus* (Tab. 4). Thus, when *A. flavicollis* were absent, *A. sylvaticus* occurred relatively more in the woodland section of the enclosure. The difference in distribution of *A. sylvaticus* dependent on whether *A. flavicollis* was present or not is significant ($p < 0.05$, and $p < 0.05$).

3.1.4. Vertical distribution in artificial habitats of a large indoor pen

As shown in Tab. 5 (Exp. IV), there is a clear difference in the choice of the two species when tested together in the pen, both with 4 and with 8 individuals of each species per test ($\chi^2 = 14.070$; $p < 0.001$ and $\chi^2 = 10.165$; $p < 0.005$, respectively): Most *A. flavicollis* nested above-ground; the difference between the number nesting above-ground and the number nesting on the ground is significant ($p < 0.05$ and $p = 0.038$, Bin.-test, respectively). On the contrary, most *A. sylvaticus* nested on the ground; the difference between the number nesting above-ground and the number nesting on the ground is significant ($p < 0.01$ and $p = 0.01$, Bin.-test, respectively).

Furthermore, there was a clear difference in the distribution of *A. sylvaticus* with and without *A. flavi-*

Tab. 4. Influence of the presence of *A. flavicollis* males on the distribution of *A. sylvaticus* males in the 150 m² outdoor enclosure (Exp. III). Results are based on trapping during the second night after introduction of the mice. The figures given for the distribution of *A. sylvaticus* when *A. flavicollis* were present, are the same as in Tab. 3.

Type of experiment	No. of individuals trapped in:	
	W–I	G
A) With *A. flav.* in the enclosure (see Tab. 3I).	2	18
B) Without *A. flav.* in the enclosure	10	14
	W–II	G
A) With *A. flav.* in the enclosure (see Tab. 3II).	6	22
B) Without *A. flav.* in the enclosure	5	3

W– I = beech-wood half of enclosure, with poor climbing and hiding possibilities.
W–II = beech-wood half of enclosure, with improved climbing and hiding possibilities.
G = grassland half of enclosure.

Tab. 5. Vertical distribution. Comparison of *A. flavicollis* and *A. sylvaticus* males as to the choice of nesting sites – 1 m above ground or down on the floor – in the 16 m^2 indoor enclosure with artificial woodland and grassland habitats (Exp. IV). The 2 × 8 *A. sylvaticus* of B) are the same as those of A-b); for these animals, the nesting sites were first recorded, when they were alone in the enclosure, and secondly after *A. flavicollis* had been added.

Type of experiment	*A. flavicollis*		*A. sylvaticus*	
	No. nesting 1 m above the ground	No. nesting down on the ground*	No. nesting 1 m above the ground	No, nesting down on the ground*
A) The two species together in the enclosure				
a) 4 *A. flav.* + 4 *A. sylv.* per test	20	8 (0 W) (8 G)	6	22 (6 W) (16 G)
b) 8 *A. flav.* + 8 *A. sylv.* per test.....	12	4 (1 W) (3 G)	3	13 (0 W) (13 G)
B) *A. sylv.* alone in the enclosure	–	–	9	7 (0 W) (7 G)
8 animals per test				

* The numbers given in brackets indicate the proportion of individuals nesting down in the "woodland" section (W) and in the "grassland" section (G).

collis ($p < 0.05$). In this respect, only results from the tests with eight individuals in each (4 + 4 or 8) are compared: 9 above/7 down on the ground, and 6 above/22 down on the ground, respectively. Thus, when *A. flavicollis* were not in the enclosure, almost equal numbers of *A. sylvaticus* nested above and on the ground, whereas when *A. flavicollis* were present and nesting above the ground – as most of them did – the majority of *A. sylvaticus* nested down on the ground.

3.2. Behaviour

3.2.1. Interaction in a large indoor pen

3.2.1.1. Interspecific interaction

Few encounters between individuals of the two species were "neutral" (Tab. 6). *A. flavicollis* was dominant in almost all of the encounters observed, and its dominance was mainly of the "passive" type: 86 "passive" against 17 "active", ($p < 0.001$) *A. sylvaticus* in general reacted with escape and hiding at the mere approach (non-aggressive) of some individual *A. flavicollis*.

3.2.1.2. Intraspecific interaction

As shown in Tab. 7, there is a significant difference between the two species with regard to the relative proportions of the categories of interaction recorded ($\chi^2 = 66.15$; $p < 0.001$). Encounters between *A. flavicollis* were distributed rather evenly into the "active", "passive" and "neutral" types. With *A. sylvaticus*, there were only a few "neutral" encounters and the "active" type of dominance prevailed. Thus, there seems to have been less intraspecific antagonism between *A. flavicollis* than between *A. sylvaticus*.

3.2.2. Activity in a new area ("exploration")

There was a difference between *A. flavicollis* and *A. sylvaticus* in the duration of activity after introduction into the test enclosure (Tab. 8). More *A. flavicollis* remained active for a longer time moving about in the whole area, while *A. sylvaticus* showed a stronger tendency to run under cover in the grassland section and stay there. *A. flavicollis* further had an inhibiting effect on *A. sylvaticus*: they increased their tendency to run under cover and remain inactive. There was also a difference in the way the two species behaved when introduced into a new area. *A. flavicollis* moved about in a more quiet and leisurely manner showing more elements of true investigation ("nosing" and "sniffing", cf. van Oortmerssen 1970). *A. sylvaticus* moved through the area in a more "timid and nervous" way, often making quick dashes from one shelter to the next. This type of behaviour was enhanced by the presence of *A. flavicollis*.

3.2.3. Climbing tendency

When introduced into a new area *A. flavicollis* climbed more readily than *A. sylvaticus* (Tab. 9); they also climbed more frequently. *A. sylvaticus* climbed less when *A. flavicollis* were present (Tabs. 5, 9).

Tab. 6. Interspecific interaction. Encounters observed between *A. flavicollis* and *A. sylvaticus* males in the 16 m^2 indoor pen in Exps. II and IV. Dominance relationship and relative occurrence of the various kinds of interaction. A total of 36 *A. flavicollis* and 37 *A. sylvaticus* were involved.

Total encounters observed	No. of encounters where	No. of encounters where	No. of "neutral" encounters
	A. flav. dominated	*A. sylv.* dominated	
130	103 { 17[1] / 86[2]	11 { 6[1] / 5[2]	16

[1] "active" [2] "passive"

Tab. 7. Intraspecific interaction. Encounters observed between *A. flavicollis* males and between *A. sylvaticus* males, respectively, in the 16 m² indoor pen in Exps II and IV. Relative occurrence of the various kinds of interaction. A total of 56 *A. flavicollis* and 64 *A.sylvaticus* were involved.

Total observed	"Active' dominance	'Passive" dominance	"Neutral"
	Encounters between *A. flav.*		
137	42	39	56
	Encounters between *A. sylv.*		
156	98	53	5

4. Discussion

4.1. General behaviour

A. flavicollis was less timid, less dependent on cover, more active than *A. sylvaticus* when exposed to new surroundings, and also showed a stronger tendency to climb. These differences in behaviour between the two *Apodemus* species are assumed to be of some relevance to their distribution in nature.

The differences I have found are very similar to those described between two subspecies of *Peromyscus*, the woodland form *P. maniculatus gracilis*, and the grassland form *P. maniculatus bairdi* (Horner 1954, Foster 1959, King et al. 1968). Thus, there is some indication that these differences might be of a more general nature distinguishing woodland- from grassland-adapted forms.

The greater climbing tendency of *A. flavicollis* is well correlated with its better anatomical adaptations for arboreal life (Heinrich 1927), its occurrence in tall trees (Borowski 1963), and in abandoned squirrel and birds' nests.

Tab. 8. Duration of exploration after introduction into a new area – the 16 m² large indoor pen – in Exp. IV. In the columns are indicated, the number of individuals active at each 5-min interval after introduction.

Min after introduction into a new area	Either species alone		Two species together	
	A. flav.	*S. sylv.*	*A. flav.*	*A. sylv.*
5	13	8	22	18
10	13	6	21	6
15	13	8	19	7
20	11	9	18	11
25	12	7	18	3
30	10	5	18	2
35	9	5	19	3
40	12	7	14	1
45	12	6	13	0
Total no. of individuals tested	16	16	24	24

4.2. Interaction

4.2.1. Interspecific interaction

Reports on the presence of one or the other species alone in a restricted place suggest the occurrence of interspecific interactions (cf. Pedersen 1961) but observations on direct encounters between the two species are few and unconvincing (Curry-Lindahl 1959).

The figures in Tab. 6, supported by indirect evidence in Tabs. 2, 4, 5, 8, and 9 suggest that *A. flavicollis* has an intimidating effect on *A. sylvaticus*. Adult males of *A. flavicollis* are larger than adult males of *A. sylvaticus*, but dominance need not always be dependent on larger size during interspecific encounters (Getz 1962, Grant 1970). My results suggest that *A. sylvaticus* is intrinsically more aggressive than *A. flavicollis*, but that *A. flavicollis* was able to dominate *A. sylvaticus* in a passive way. This result could not have been predicted since in interspecific encounters the more aggressive species is often dominant (King 1957, Murie 1971) and furthermore in encounters with *Clethrionomys glareolus*, *A. flavicollis* was found to be quite aggressive (Andrzejewski and Olszewski 1963); in the last-mentioned experiments, however, the animals were placed in a more competitive situation by the provision of single baited traps.

The observation that *A. sylvaticus* tended to hide and stay inactive while *A. flavicollis* moved about in the pen is in accordance with other observations concerning time segregation between dominant and subordinate rodents, both interspecifically (Andrzejewski and Olszewski op. cit.) and intraspecifically (Brown 1969, Bovet 1972). Another way of reducing encounters between the two *Apodemus* species would be the tendency to segregate vertically.

Tab. 9. Climbing. In the columns are indicated the cumulative number of individuals having climbed to the top of the lattice work constituting the artificial woodland of Exp. IV within the min-intervals chosen.

Min after introduction into the test area	Either species alone		Two species together	
	A. flav.	*A. sylv.*	*A. flav.*	*A. sylv.*
1	2	0	8	1
5	6	0	12	1
10	8	1	12	1
15	9	1	13	1
20	11	1	15	1
25	11	2	16	1
30	11	3	16	1
35	11	3	16	1
40	11	4	18	1
45	11	4	19	1
50	11	5	20	1
Total no. of individuals tested	16	16	24	24

4.2.2. *Intraspecific interaction*

In the present investigation, *A. flavicollis* were found to be less aggressive towards members of their own species than *A. sylvaticus*. In the area from which the test animals were derived. *A. sylvaticus* was more abundant than *A. flavicollis*, and the difference in aggressiveness could be the result of this difference in population sizes. The finding of a rather large number of non-aggressive meetings between *A. flavicollis* individuals is consistent, however, with observations made by Zimmermann (1956) and with data from Andrzejewski & Olszewski (op. cit.). As to *A. sylvaticus*, Brown (1966) and Bovet (op. cit.) agree that individuals of this species are fairly aggressive. Seasonal changes in aggressiveness (Sadleir 1965, and Brown op. cit.), or periodical variations (Bovet op. cit.) are not likely to have influenced the present results, since observations were made within the breeding period of both species and on animals derived from many different natural groups.

4.3. Choices in the two-habitats system

Generally speaking, *A. flavicollis* occurred almost evenly in both habitats offered, whereas *A. sylvaticus* preferred the grassland section. In the wild, according to trapping results within the area from which the test animals were derived (Hansson, pers. comm.) *A. flavicollis* occurs rather exclusively in woodland, whereas *A. sylvaticus* occurs in both woodland and grassland. Variations from this pattern are reported by other authors, mainly as to the degree of ecological separation of the two species (e.g. Mohr, 1931, Hamar et al. 1966, Curry-Lindahl op. cit.). In the test enclosures both species occurred relatively more in the grassland habitat; thus, there is apparently a displacement of the total "species equilibrium" from woodland towards grassland. In the light of this, it appears that the distinct favouring of the woodland section of such enclosures in similar experiments has been difficult to obtain even in woodland forms (Grant op. cit.), and in woodland"imprinted" forms (Wecker op. cit.). On the other hand, preference for the grassland section was easily demonstrated in *Microtus* (Grant op. cit.) and in the grassland form *Peromyscus maniculatus bairdi* and different kinds of its first generation offspring (Wecker op. cit.). Thus, under similar experimental conditions "woodland" types apparently tend to distribute almost evenly in both woodland and grassland, whereas the "grassland" types occur more in the grassland section.

A general increase in hiding tendency must be expected as an artefact of the experimental situation. This will imply a general rise in grassland occurrence, as most small rodent species may find better overall shelter there. Further, differences in timidity when exposed to new surroundings found between the two *Apodemus* species may partly explain why *A. flavicollis* occurred relatively more outside the sheltering vegetation of the grassland sections than *A. sylvaticus*. The stronger tendency to climb in *A. flavicollis* no doubt also contributed to the greater occurrence of this species in the woodland.

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I - APPENDIX

COMPARATIVE STUDY ON BEHAVIOR IN WOODLAND-GRASSLAND ENCLOSURES

In the experimental study on habitat selection (I), A.flavicollis occurred about evenly in both forest and grassland sections, while A.sylvaticus occurred more often in grassland. A.flavicollis also showed a stronger tendency to climb. The distribution pattern found in the enclosure experiments did not correspond to that mostly found in the natural situation (see e.g. Hoffmeyer & Hansson 1974). There appeared to be a displacement of the natural distribution pattern (Fig. 1.1) towards grassland in the enclosure situation (Fig. 1.2).

Similar 2-habitat enclosure experiments with other small rodent pairs (Harris 1958, Wecker 1963 and Grant 1970) and my own observations on Microtus and Clethrionomys species, revealed striking parallels in behavior among the forest types (Peromyscus maniculatus gracilis and Clethrionomys) on one side, and among the field types (Peromyscus maniculatus bairdi and Microtus) on the other. In all these enclosure experiments the forest types occurred about equally in both habitat sections (enclosure halves), whereas the field types kept to the grassland section. This indicates convergence in behavior among forest forms respectively among "open-land" forms. The similarity of A.sylvaticus, enclosure behavior to that of the grassland form P.man.bairdi and to that of Microtus should be noticed.

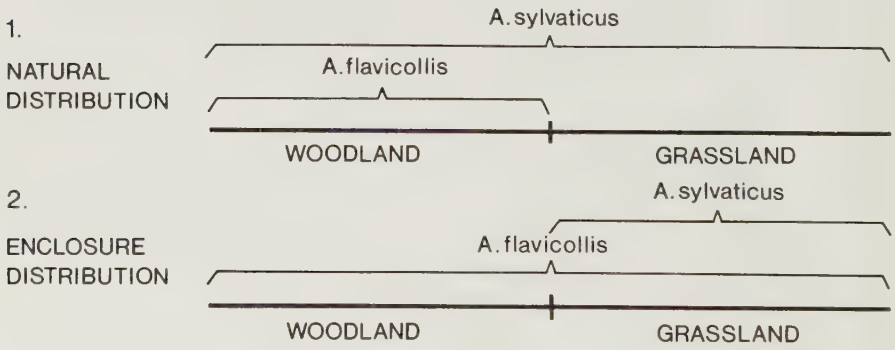

Fig. 1. Increase in grassland occurrence of both Apodemus species during short-term enclosure experiments. Woodland = beech forest with sparse ground vegetation cover. Grassland = area with rich ground vegetation cover (mainly Dactylus glomerata).

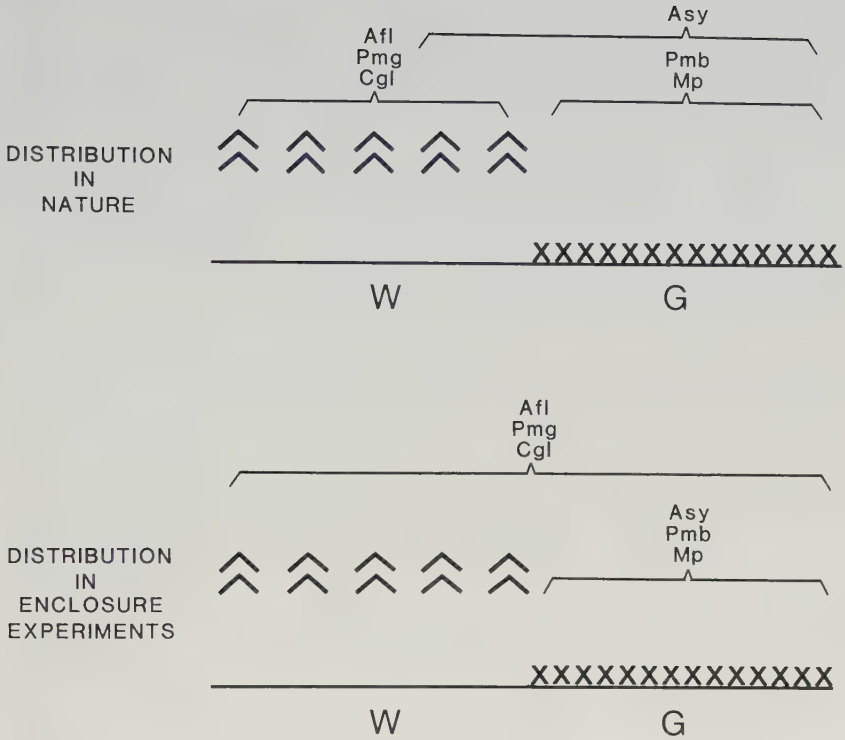

Fig. 2. Similarity in behavior between different woodland-adapted small rodents (left) and between different grassland adapted ones (right) in short-term enclosure experiments. Peromyscus behavior deduced from data of Harris (1958) and Wecker (1963). Clethrionomys and Microtus behavior deduced from data of Grant (1970). (References in I.)

How can the discrepancies in the results of field studies and experiments be explained?

1. The difference in distribution of the two Apodemus species in the two-habitat (woodland-grassland) enclosures might be determined largely by a difference in their need of cover at ground level, such cover being available in the grassland section only.

2. The "displacement" of the natural distribution pattern towards grassland in the enclosure experiments (Fig. 1.1 vs 1.2) was in part due to an increase in seeking shelter by both species as they were in an unfamiliar area.

Thus, apart from expressing an intrinsic preference for a given vegetation type (habitat selection), the occurrence of a small rodent in the grassland section during short-term enclosure experiments, similar to those of Harris (1958), Wecker (1963) and

Grant (1970) might be due partly to an increased fugitive response in an unfamiliar environment.

The observed differences in behavior between the two Apodemus may reflect adaptation to different habitat conditions, and could operate in two ways to determine the distribution of each species in enclosure experiments: 1. Directly, by habitat selection, making the individuals of either species actively chose different structural features of environment, e.g. trees (A.flavicollis) vs ground cover (A.sylvaticus), and 2. Indirectly, through differences between the two species in predator avoidance and defence efficiency (see p.35), reflecting on their behavior in novel environments (the relative degrees of timidity). In other animal species differences in timidity are correlated with differences in defence efficiency (Tinbergen 1973). This is also primarily caused by adaptation to different habitats, because a habitat which offers little protection makes alternative defence adaptations necessary. According to results of Erlinge et al. (1974) exposed A.sylvaticus are more susceptible to predation than A.flavicollis, and my own observations suggest that A.flavicollis have evolved more elaborate defence devices than A.sylvaticus (see below).

TABLE 1

Climbing and utilization of ground cover. Animals tested individually. N_x = number of animals tested. Afl = A.flavicollis; Asy = A.sylvaticus. [x] Figures from two test series with different animals and different % of ground cover.

	N		mean values		P
	Asy	Afl	Asy	Afl	
Climbing frequency (test period 30 min.)	19	19	6	15	0.002
Latency to leave a cover on the ground (secs)	20	24	263	151	0.02
Cumulated time spent under cover (secs)[x]	20 16	20 18	319 571	162 284	0.002 0.002

(Statistical method: Mann-Whitney U test, two-tailed)

DIFFERENCES IN DEFENCE BETWEEN A.FLAVICOLLIS AND A.SYLVATICUS

1. Avoidance: Sudden strong jumps or climbing up trees are efficient escape reactions most characteristic of A.flavicollis. A.sylvaticus depend more on just hiding, mostly running to some shelter on the ground. A.flavicollis also have more elaborate camouflage devices against visual predators: Longer lasting immobility reactions (freezing), which in combination with the reddish-brown fur colour seem especially well adapted camouflage on open ground areas of deciduous forest. The long-lasting immobility also protects against other predators for which e.g. rattling noise caused by movement in dead leaves could be used for prey--detection. Conversely, the fugitive behavior of A.sylvaticus is better adapted for habitats with dense ground vegetation. Their smaller body size and more greyish fur colour allow them to be concealed by this kind of vegetation.

2. Active defence: The startling effect of the sudden and violent escape movements of A.flavicollis constitutes another type of efficient defence (see e.g. Edmunds 1974). Furthermore, A.flavicollis have a stronger and more damaging bite, which probably is adapted primarily for other requirements such as feeding on hard seeds or constructing burrows where gnawing wood is necessary.

The more elaborate and efficient defence devices of A.flavicollis may help explain why adult individuals of this species were generally less cautious in new environment and less reluctant to enter open space.

APODEMUS FLAVICOLLIS ASSOCIATION WITH LARGE TREES

Apodemus flavicollis are frequently found in association with large trees (beech, oak, ash).

This is probably due to habitat selection resulting from long term evolutionary adaptations to mature deciduous forest and to the type of food - mainly energy-rich oily seeds - connected with these trees.

The aggregation of A.flavicollis on the food patches presented by these large trees then may have favoured other evolutionary adaptations, such as greater climbing tendency and skills (Löhrl 1938, Heinrich 1958, Borowski et al. 1963, Holišova 1969). This climbing may primarily have evolved for predator escape (incl. the nesting in hollow trees), and to exploit alternative food

sources. The large seed stores on which A.flavicollis are very dependent for overwintering must have favoured strong site attachment in these animals. The consequence then was that they had to explore alternative close-by food sources whenever the main stores became exhausted. Also, the evolution of better climbing skills by A.flavicollis probably was advantageous since there would be less competition for tree seeds from other more ground-dwelling granivorous rodents.

Finally the adaptations for climbing inevitably were accompanied by a reduced capacity for digging burrows - a consequence of being separated from the ground. Therefore, the natural crevices found in connection with large mature trees (between the roots, or in the trunks) became used by A.flavicollis as refuges.

As a supplement it is interesting to note that in other geographical regions where A.sylvaticus coexist in forest habitat with another closely related Apodemus species, the latter is reported to climb trees more readily than A.sylvaticus. This is the case with Apodemus mystacinus in Israel (Granot 1978).

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Experiments on the selection of food and foraging site by the mice Apodemus sylvaticus (Linné, 1758) and A. flavicollis (Melchior, 1834)

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With 1 Figure

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1. Introduction

The problem of the ecological separation of the two closely related species of mice *Apodemus sylvaticus (Asy)* and *A. flavicollis (Afl)* is still rather unsolved. Their respective habitat distributions vary with latitude, year and season. In Sweden, both live in woodland, but *Afl* is more restricted to this habitat than *Asy*, which also live in open-field areas.

Among the causes of differences in the habitat distribution of small mammals, – climate, vegetation type, food habits and social relations are the most important (DRICKAMER 1972). Concerning vegetation type, some experiments on habitat selection, showed differences between the two *Apodemus* species in their distribution, and direct observation of the animals suggested that behavioural factors play a rôle (HOFFMEYER 1973, and HOFFMEYER in prep.). The relations between the two species were also studied and were found to have an effect as well. This experimental result was later supported by studies of free-living populations (HOFFMEYER & HANSSON 1974). Food should also be considered as a possible cause of habitat segre-

gation. HEINRICH (1951) suggested that *Afl* have a one-sided preference for oily tree seeds, whereas *Asy* have a special taste for starchy seeds of corn and grass. However, according to PFEIFFER & NIETHAMMER (1972) there seems to exist no specific differences in food choice between *Asy* and *Afl* and HANSSON (1971) also just classifies both as granivorous species.

The present paper deals with the comparison of *Asy* and *Afl* concerning selection between two types of seeds (beech-, *Fagus silvatica* and grass-, *Dactylus glomerata)* when these seeds are provided in a way simulating the natural conditions of late summer/early autumn, where both types are ripe and still sitting on the respective plants. Furthermore, an experiment is made concerning the selection between feeding sites – in woodland or grassland, and in woodland – on the ground or up on the trees.

2. Material & Methods

2.1. *Animals:* A total of 112 *Asy* and 104 *Afl* were tested. Each individual was used only once.

All the wild captured mice were from the Revinge area in southernmost Sweden, where the two species occur together and where their habitat distribution was being studied as well. They were trapped in late august – beginning of september simultaneously with the execution of the experiments. The *Afl* were mainly caught in beech forest and the *Asy* in the adjacent abandoned fields, grassland dominated by *Dactylis glomerata.* However, several of both species were trapped close to or at the habitat border. For the food selection experiments, they were kept in captivity for only 1–2 days before use. They were held individually in standard mouse cages ($40 \times 24 \times 15$ cm), provided with nesting material, a small nesting cover, mouse pellets (EWOS) and drinking water freely available, in a special mouse house at the natural day-night cycle. The laboratory bred mice were born by wild captured mothers from the same area, which were pregnant at the time of trapping. They were isolated from their mothers at weaning. Some were fed with normal mouse pellets; others were fed with mash = a mixture of crushed mouse pellets and chicken food. The laboratory born mice were tested at the age of 8–10 weeks, and care was taken that the wild trapped used were of about the same age and size.

2.2. *Experiments on food selection:* Beech mast *(Fagus silvatica)* and grass seeds *(Dactylus glomerata)* were provided in a way simulating natural conditions of late summer, early autumn, where both are still sitting on the respective plants. The items were collected immediately before use. The beech mast was obtained by cutting down branches on which the ripe seeds were sitting in the spathes still closed. The bursting open of the spathes was prevented by keeping the branches in large plastic bags provided with pieces of moist cotton wool to maintain air humidity. Whole grass straws with spikes at the top were also kept in plastic bags until use.

The test cages were mainly large plastic barels (ϕ = 60 cm; h = 40 cm) with a top made of the same material and with holes for ventilation. In the pilot experiment, however, another type of cages were used to allow behaviour observations. These were $120 \times 60 \times 60$ cm large cages, made of Al-sheet and with a front glass. All the test cages had a nest box, an artificial "tree", a grass turf, and the rest of the floor covered with wood shavings.

All mice were tested individually, in series of 6–7 at a time, – the two species alternately. In the pilot experiment (Tabs. 1 and 2) however, where as mentioned an attempt was made to study the feeding behaviour of the mice, 2 individuals were introduced into each test cage at a time, in order to augment chances of seing any.

In each test cage a number of the two above mentioned types of food were placed in the following ways: 1) The beech mast in still closed spathes on branches: Half of the number of spathes given per test were attached on the top of the "tree" of the cage,

Fig. 1: Different degrees of exploitation of grass spikes *(Dactylus glomerata)* by mice. The scale 0–4 is used to designate increasing grades of exploitation. Picture no. 4, however, does not quite show the heaviest grade "4".

the other half placed on the cage floor. 2) The grass seeds still on spikes: Half of the number of spikes given per test were left on the top of straws, which were reinforced by wire at the base and stuck into the grass turf of the cage to stand erect; the other half of the spikes were cut off the straws and attached with tape to a small piece of board (Fig. 1) and placed on the cage floor in a dish to avoid scattering of the seeds.

Table 1: Food choice by wild trapped *Asy* (Pilot Experiment).

		Beech Spathes	Grass Spikes	
No.	Sex	No. taken	No. taken	Exploitation
1	♂+♂	3/6	1/3	3
2	♂+♂	2/6	3/3	3
3	♂+♂	0/6	0/3	1
4	♂+♀	0/6	2.5/3	3
5	♀+♀	0/6	2/3	4
6	♂+♂	2·8	2/3	2
7	♂+♀	0·8	1/3	1
8	♂+♂	0/8	0/3	1
9	♂+♀	0/8	0/3	1
10	♀+♀	0/8	0/3	2
11	♂+♂	7/8	3/3	0
Total taken		16/78 (21 %)	14.5/33 (44 %)	

The ratios refer to the number of units (spathes or spikes) taken/number of units offered per test, during 1 night. Exploitations refers to the scale 0 – 4 (Fig. 1).

Table 2: Food choice by wild trapped *Afl* (Pilot Experiment). For further information see the text of Tab. 1.

		Beech Spathes	Grass Spikes	
No.	Sex	No. taken	No. taken	Exploitation
1	♂+♂	6/6	0/3	0
2	♂+♀	6/6	3/3	2
3	♂+♂	8/8	0/3	1
4	♂+♀	9/9	2/3	1
5	♀+♀	8/8	0/3	0
6	♂+♂	1/8	0/3	2
7	♂+♂	7/8	0/3	0
8	♂+♂	8/8	0/3	1
9	♂+♀	8/8	0/3	0
10	♂+♀	8/8	0/3	0
11	♂+♂	8/8	0/3	0
Total taken		77/85 (91 %)	5/33 (15 %)	

Furthermore, drinking water and a small amount of standard food were provided. The latter to prevent the mice from starving in case they would take none of the natural food items provided.

The food items were placed in the cages as described above, at the fall of night, – before the start of the mice' activity period, and registrations were made on the following morning. If not otherwise stated, results are from the 1st test night only, to avoid changes due to the mice' experience with the food objects provided. The following was registered:

1. for beech: a) the number of spathes, which had been detached (taken) from the branches; b) the number of spathes, which had been gnawed and c) the number of spathes, which the mice had penetrated (= gnawed sufficiently) to get the seeds out. 2. for grass: a) the number of spikes, which had been taken from the erect straws; b) to which degrees the spikes on the ground had been exploited. For the latter, a scale 0–4 was used (Fig. 1). Furthermore, in one test series (Tab. 6) were noted, the number of straws, which had been: 1) cut at the top, below the spike, while erect; 2) cut at the basis; 3) bent or 4) left intact.

The following test series were made: 1. With wild trapped animals. Pilot experiment (Tabs. 1 and 2); – Experiment I (Tabs. 3 and 4); and Experiment II (Tab. 5) which only concerned the exploitation of beech spathes. 2. With laboratory born animals. Experi-

Table 3: Food choice by wild trapped *Asy* (Experiment I).

			Beech Spathes			Grass Spikes	
No.	Sex	Weight g	No. taken	No. gnawed	No. penetrated	No. taken	Exploitation
1	♂	19	6/6	5/6	0/6	3/3	4
2	♂	21	5/6	5/6	1/6	3/3	1
3	♀	20	0/6	0/6	0/6	2.5/3	1
4	♀	20	6/6	4/6	2/6	3/3	4
5	♂	17	6/6	6/6	2/6	3/3	4
6	♂	22	0/6	2/6	0/6	2.5/3	2
7	♀	22	1/6	1/6	0/6	3/3	3
8	♀	23	4/6	2/6	0/6	1/3	3
9	♂	17	6/6	4/6	4/6	0.5/3	0
10	♂	17	6/6	4/6	3/6	0/3	0
11	♂	16	3/6	0/6	0/6	0/3	0
12	♂	19	1/6	0/6	0/6	3/3	4
13	♀	22	3/6	0/6	0/6	3/3	0
14	♀	18	6/6	3/6	3/6	2/3	3
15	♂	20	3/6	3/6	3/6	0/3	2
16	♂	22	5/6	5/6	0/6	2.5/3	4
17	♂	22	4/6	1/6	1/6	3/3	4
18	♂	23	5/6	1/6	0/6	2.5/3	3
Total			70/108 (65 %)	46/108 (43 %)	19/108 (18 %)	37.5/54 (69 %)	

The ratios refer to the number of units (spathes or spikes) / number of units offered per test, during 1 night. "Taken" means detached from the plant (branch or straw); "gnawed" means that the spathe bears traces of being gnawed, yet not sufficiently to get the seeds out; "penetrated" means gnawed sufficiently to get the seeds out. Exploitation (grass spikes) refers to the scale 0 – 4 (Fig. 1).

Table 4: Food choice by wild trapped *Afl* (Experiment I).

			Beech Spathes			Grass Spikes	
No.	Sex	Weight g	No. taken	No. gnawed	No. penetrated	No. taken	Exploitation
1	♂	28	7/8	3/8	0/8	1.5/3	1
2	♂	28	0/8	0/8	0/8	0/3	3
3	♂	26	6/8	6/8	5/8	1.5/3	3
4	♂	30	4/8	4/8	3/8	0/3	0
5	♂	33	8/8	7/8	7/8	1/3	2
6	♂	30	8/8	8/8	8/8	2/3	0
7	♀	28	8/8	7/8	3/8	0.5/3	4
8	♀	28	8/8	8/8	6/8	1/3	0
9	♀	26	8/8	8/8	8/8	1.5/3	0
10	♂	40	8/8	8/8	8/8	0/3	0
11	♂	40	8/8	8/8	8/8	2/2	2
12	♂	33	6/8	3/8	2/8	1.5/3	3
13	♀	27	8/8	8/8	8/8	1.5/3	0
14	♀	37	8/8	8/8	8/8	1/3	0
15	♂	26	8/8	8/8	7/8	1.5/3	1
16	♀	22	8/8	8/8	7/8	2.5/3	4
17	♂	30	3/8	3/8	2/8	1.5/3	4
18	♂	33	8/8	8/8	8/8	1/3	1
Total			122/144 (85 %)	113/144 (78 %)	98/144 (68 %)	21.5/54 (40 %)	

The ratios refer to the number of units (spathes or spikes) / number of units offered per test, during 1 night. For further information, see the text of Tab. 3.

ment III (Tabs. 7 and 8) with animals bred on mash, and Experiment IV (Tab. 9) with animals bred on pellets.

2.3. *Experiments on the selection of foraging site:* The mice were given the choice between foraging places, provided with identical food (standard mouse pellets), in grassland – woodland, and upon trees – on the ground of the woodland.

For this purpose, simulated natural habitats were arranged in large indoor enclosures (4×2×2 m each). The "woodland" contained four trees (abt. 1.2 m high) supporting

Table 5: Comparison of wild trapped *Asy* and *Afl* (Experiment II), concerning only the exploitation of beech mast in spathes.

Apodemus sylvaticus					Apodemus flavicollis				
			Beech Spathes					Beech Spathes	
No.	Sex	Weight g	No. gnawed	No. penetrated	No.	Sex	Weight g	No. gnawed	No. penetrated
1	♂	21	0/12	0/12	1	♂	35	7/12	6/12
2	♂	18	0/12	0/12	2	♂	39	11/12	9/12
3	♂	19	1/12	0/12	3	♂	42	4/12	1/12
4	♂	17	1/12	1/12	4	♂	37	9/12	6/12
5	♂	22	5/12	0/12	5	♂	38	4/12	3/12
6	♂	22	3/12	0/12	6	♂	42	6/12	4/12
7	♂	20	0/12	0/12	7	♂	32	4/12	0/12
8	♂	17	0/12	0/12	8	♂	36	10/12	5/12
9	♂	17	0/12	0/12	9	♂	37	6/12	4/12
10	♂	16	1/12	0/12	10	♂	35	7/12	7/12
11	♂	17	0/12	0/12	11	♂	26	7/12	2/12
12	♂	17	0/12	0/12	12	♀	29	0/12	0/12
13	♀	16	7/12	0/12	13	♀	30	8/12	8/12
14	♀	17	0/12	0/12	14	♂	34	1/12	0/12
15	♂	16	0/12	0/12	15	♂	34	6/12	6/12
16	♂	16	1/12	0/12					
17	♂	15	4/12	2/12					
Total			23/204 (11 %)	3/204 (1 %)	Total			90/180 (50 %)	61/180 (34 %)

No grass spikes were provided in this experiment. The ratios refer to the number of spathes gnawed (see the text of Table 3) or penetrated / number of spathes offered per test, during 1 night.

Table 6: Comparison of *Asy* and *Afl* concerning the taking of grass spikes from erect straws.

		Number of straws which were				
		intact	bent	cut at the basis	cut at the top	totals
A. flavicollis wild trapped	n = 10	27	9	11	13	60
A. sylvaticus wild trapped	n = 11	21	3	2	46	72
A. sylvaticus laboratory bred	n = 9	45	0	0	9	54

The results are from two consecutive nights/test. In one of the tests with wild trapped *Asy*, 6 instead of the usual 3 straws/night were provided.

Table 7: Food choice by laboratory born *Asy*, bred on mash (Experiment III). For further information, see the text of Tab. 3.

			Beech Spathes			Grass Spikes	
No.	Sex	Weight g	No. taken	No. gnawed	No. penetrated	No. taken	Exploitation
1	♂	29	0/6	1/6	0/6	2.5/3	4
2	♂	26	1/6	0/6	0/6	0.5/3	0
3	♀	20	1/6	1/6	0/6	1.5/3	4
4	♀	24	2/6	0/6	0/6	2.5/3	2
5	♂	22	0/6	0/6	0/6	3/3	2
6	♂	27	6/6	3/6	0/6	2.5/3	4
7	♂	22	1/6	3/6	1/6	1.5/3	4
8	♀	19	0/6	0/6	0/6	2.5/3	3
9	♂	19	0/6	0/6	0/6	3/3	4
10	♀	17	4/6	2/6	0/6	3/3	4
11	♂	18	0/6	0/6	0/6	3/3	3
12	♂	20	0/6	0/6	0/6	2/3	4
13	♂	24	0/6	0/6	0/6	2.5/3	4
14	♂	20	0/6	4/6	0/6	1.5/3	4
15	♀	16	0/6	0/6	0/6	2.5/3	4
Total			15/90 (17 %)	14/90 (16 %)	1/90 (1 %)	33.5/45 (74 %)	

a platform made of wire mesh on which fresh beech branches were placed at every new test. The floor was covered with a layer of soil, dead beech leaves, some logs and branches. The "grassland" was made of large turves of grass (mainly *Dactylus glomerata)* placed closely together on a layer of soil. All the grass spikes were removed. The grass was watered regularly to remain fresh. Nest boxes were placed on the ground of each habitat, as well as on the trees of the woodland. A drinking bottle was hung over the habitat boundary.

On each foraging site was placed a glass filled with pellets (standard food EWOS) and covered by wire netting to prevent the mice' hoarding the pellets. These food glasses stood on plastic plates to facilitate cleaning between the tests. Before every new test, the food was renewed and food glasses, net cover, and base plates were carefully cleaned with concentrated dish solution in hot water, rinsed and dried in a heated room. Furthermore, vegetation around every foraging site was replaced by fresh. The mouse pellets used in the experiment were always kept in the test room to minimize alterations in weight due to differences in air humidity.

All the mice were tested individually. Each one was introduced into the enclosure in the evening and for the 1st night left to explore the rather large new area and get acquainted with the foraging sites. Preference of foraging site was assessed by weighing the food glasses with contents before and after the 2nd test night. It was assumed that

Table 8: Food choice by laboratory born *Afl*, bred on mash (Experiment III). For further information, see the text of Tab. 3.

			Beech Spathes			Grass Spikes	
No.	Sex	Weight g	No. taken	No. gnawed	No. penetrated	No. taken	Exploitation
1	♀	28	8/8	8/8	1/8	1.5/3	2
2	♀	27	7/8	3/8	1/8	0/3	0
3	♀	26	0/8	0/8	0/8	0/3	0
4	♂	28	3/8	4/8	0/8	1/3	0
5	♂	37	0/8	0/8	0/8	0/3	0
6	♀	25	0/8	0/8	0/8	0/3	1
7	♂	32	3/8	3/8	0/8	0/3	1
8	♂	32	7/8	4/8	2/8	2/3	1
9	♀	26	0/8	0/8	0/8	0/3	2
10	♀	29	8/8	2/8	2/8	0/3	1
11	♂	29	0/8	0/8	0/8	0/3	0
12	♂	30	1/8	1/8	1/8	1/3	1
13	♂	35	0/8	0/8	0/8	1.5/3	3
14	♀	25	0/8	0/8	0/8	0/3	0
Total			37/112 (33 %)	25/112 (22 %)	7/112 (6 %)	7/42 (17 %)	

Table 9: Food choice by laboratory born *Asy*, bred on pellets (Experiment IV). For further information, see the text of Tab. 1.

		Beech Spathes	Grass Spikes	
No.	Sex	No. taken	No. taken	Exploitation
1	♀	8/10	0/3	1
2	♀	3/10	0/3	0
3	♂	3/10	2/3	1
4	♂	2/10	0/3	1
5	♂	1/6	0/3	0
6	♀	6/6	1/3	0
7	♂	1/6	0/3	0
8	♀	2/6	0/3	1
9	♂	2/6	0/3	0
Total taken		28/70 (40 %)	3/27 (11 %)	

the relative proportions of standard food eaten at the respective sites would be correlated with the times spent there, and thus would indicate preference.

The following test series were made: Experiment V (Tab. 10–V) with foraging sites in two habitats – grassland and woodland, and upon trees as well as on the ground of the woodland; Experiment VI (Tab. 10–VI) with only a woodland habitat and foraging sites upon trees and on the ground. Finally a series principally made for the study of habitat selection provided a supplementary occasion for comparison of choices between feeding sites in grassland and woodland, when there were none upon the trees of the woodland (Tab. 10–VII).

2.4. *Statistical treatment:* The experimental procedure was planned and carried through with an extensive knowledge in advance of the behaviour of the two species, – however without sufficient consideration to the subsequent statistical treatment of the results.

In the statistical tests, the unit is always the individual animal, and not the food type. However, at the bottom of most of the tables are given in numbers and percentages the amounts of food type taken or exploited. This is to permit quick comparisons, and should not be subject to statistical treatments.

The possibility of correlations between amounts of beech- and grass-items taken (or exploited) was investigated. There was no trend. About half of the correlations were positive, and the other half negative. None of them, however, was statistically significant (X^2-test, or Spearmann-rank correlation test). It was therefore concluded that the results from the two species could be compared and tested for each food type (beech or grass) separately, between the series.

In some of the series (Tabs. 3, 4, 7 and 8) the two species were offered a different number of beech spathes (*Asy* received 6/test and *Afl* 8/test). Therefore, neither the numbers nor the ratios taken (or exploited) are well suited for a statistical treatment. The best procedure, however, seemed to be dividing the material into two groups: e. g. "all beech spathes taken" / "no beech spathes taken". In that case a X^2-test may be applied in a meaningful way.

Table 10: Selection of feeding site (Experiments V, VI and VII).

Experiment	Species	no. of individuals tested	no. of individuals having fed most in		percentage of total amount of food, eaten in	
			grassland	woodland	grassland	woodland
–1	Asy	16	13	3	69	31
	Afl	14	4	10	42	58
V			woodland ground	woodland trees	woodland ground	woodland trees
–2	Asy	13	9	4	17	14
	Afl	13	8	5	30	28
			woodland ground	woodland trees	woodland ground	woodland trees
VI	Asy	14	10	4	65	35
	Afl	14	11	3	76	24
			grassland	woodland	grassland	woodland
VII	Asy	18	12	6	69	31
	Afl	21	14	7	57	43

Food was identical (standard mouse pellets) on all the sites. In Exp. V, the mice could chose between sites in "grassland" and "woodland", and within the woodland between sites on the ground and on the trees. For (V–2) only those individuals which did feed in "woodland" have been counted (for *Asy*, 13 of 16 tested, and for *Afl*, 13 of 14 tested). In Exp. VI, the mice were tested in "woodland" only, concerning their choice between upper and ground feeding sites. VII, figures are from an experiment on habitat selection with no feeding sites available on the trees of the woodland.

3. Results

3.1. *Selection and exploitation of seeds:* 3.1.1.

Comparison of the wild trapped *Asy* and *Afl* (Tabs. 1–6). Both in the pilot experiment (Tabs. 1 and 2) and in the real experiment (Tabs. 3 and 4), there was a difference between the two species as to the food type taken. *Afl* took more beech than *Asy* and *Asy* took more grass than *Afl*.

Beech taken: – If grouping the material in the categories "all taken" / "not all taken", the differences between the two *Apodemus* species are statistically significant in both experiments ($p < 0.001$, Tabs. 1 and 2; $0.025 < p < 0.05$, Tabs. 3 and 4; X^2-test).

Grass taken: – Also here both differences are statistically significant ($0.02 < p < 0.05$, Tabs. 1 and 2; $0.001 < p < 0.005$, Tabs. 3 and 4; Kolmogorov-Smirnov two-sample test).

Furthermore, there was a difference between the two species as to the exploitation of the two types of seeds (Tabs. 3, 4 and 5). The grouping "all exploited"/"not all exploited" was made, and the X^2-test applied. More *Afl* than *Asy* gnawed and penetrated the beech spathes ($p = 0.001$, Tabs. 3 and 4; $0.001 < p < 0.01$, Tab. 5). On the other side, the grass spikes were generally exploited to higher degrees by *Asy* than by *Afl*. The difference between the two species in exploitation of the grass spikes was statistically significant, $0.001 < p < 0.01$, for Tabs. 1 and 2, Kolmogorov-Smirnov test, but not for Tabs. 3 and 4. In the exploitation of grass, *Asy* also showed a stronger tendency than *Afl* to climb to the top of erect straws for reaching the spikes (Tab. 6).

3.1.2. Comparison of laboratory bred *Asy* and *Afl* (Tabs. 7, 8 and 9). When born in the laboratory and bred on identical food (mash), the two species showed fainter differences but in the same direction as found betweeen the wild trapped. These differences were not all statistically significant. *Afl* just tended to take and exploit more beech than *Asy*, whereas *Asy* clearly took and exploited more grass than *Afl* ($p = 0.001$ for spikes taken, and $p = 0.001$ for exploitation, Kolmogorov-Smirnov test).

If comparing the Tabs. 7 and 9, it appears that the *Asy* which had been bred on pellets instead of on mash showed a relative preference for beech over grass. The number of beech spathes taken is significantly higher in the pellet-group ($0.001 < p < 0.01$, X^2test, using the grouping "no taken"/"at least one taken"). On the other hand, grass spikes were taken and exploited significantly more in the mash-group ($p = 0.001$, and $p < 0.001$, Kolmogorov-Smirnov two sample test). The lack of interest for grass seeds in *Asy* bred on pellets also resorts from Tab. 6. In this group, almost no spikes were taken from the erect straws.

3.1.3. Comparison of the wild trapped with the laboratory bred (Tabs. 3, 4, 7, 8 and 9). In both *Asy* and *Afl* the wild trapped took more beech spathes than their laboratory bred conspecifics which had been fed with mash ($0.001 < p < 0.005$; $-0.001 > p < 0.005$, Kolmogorov-Smirnov test). Concerning exploitation of beech (however) there were no significant differences in *Asy* although there was a tendency towards more exploitation by the wild trapped. In *Afl* on the other hand, the exploitation by the wild trapped was significantly higher, both in the case of beech spathes gnawed, and spathes penetrated ($0.005 < p < 0.01$; $p < 0.001$, Kolmogorov-Smirnov test).

The *Asy* fed with pellets tended to take about the same amount of beech spathes as the wild trapped *Asy*, however no statistical test has been carried out in this case because of the heterogeneity of the material.

Concerning the grass spikes, the numbers taken, and the degrees of exploitation were insignificantly higher in the mash bred *Asy* compared with the wild trapped of this species. In the pellet bred *Asy*, however, numbers taken, and exploitation were signifi-

cantly lower compared with the wild trapped ($p = 0.025$; $p = 0.01$, Kolmogorov-Smirnov test). In *Afl*, the number of grass spikes *taken* was just significantly higher in the wild trapped compared with the mash bred ($p = 0.05$, Kolmogorov-Smirnov test), and the trend was the same concerning exploitation, however not statistically significant.

3.1.4. Correlation between body weights and exploitation of beech in spathes. By means of X^2-tests and especially Spearmann rank correlation coefficient (with subsequent t-tests), the possible correlation between body weight of the test subjects and the number of spathes taken and/or penetrated by them was investigated. 12 examples concerning both species, both types of food, and the categories "taken" and "exploited" were examined. The correlation was negative 6 times, zero 1 time and positive 5 times. 7 correlation coefficients were between -0.41 and $+0.35$; none was statistically significant. In *Afl*, the correlation with regard to beech penetration was $+0.24$ (Tab. 4), and $+0.35$ (Tab. 5). In Table 8, the mean weight of the 9 *Afl* penetrating no spathe was exactly the same (29.2 g) as the mean weight of the 5 *Afl* which did penetrate at least 1 spathe.

Obviously, there was not within either species any consistent correlation trend between body weight and food taken or exploited. The same seems to be the case concerning sex and food.

3.2. *Selection of feeding sites in simulated natural habitats (Tab. 10):* In the choice situation between woodland and grassland, and with the feeding sites of the woodland disposed both up on the trees and on the ground, the two *Apodemus* species differed as to where they prefered to feed (Tab. 10, V–1). *Asy* prefered to feed in the grassland section of the enclosure, while *Afl* prefered to feed in the woodland section. The difference is highly significant ($0.001 < p < 0.01$, X^2-test). In the other experiment in which food was not provided on the trees of the woodland, and where moreover climbing possibilities were scarce, *Afl* like *Asy* prefered to feed in the grassland section (Tab. 10, VII).

Within woodland only, both *Apodemus* species prefered to feed on the ground. This was the case in the experiment with only woodland available to the mice (Tab. 10, VI), but also in the woodland/grassland choice experiment (Tab. 10, V–2).

4. Discussion

In the present investigation clear interspecific differences were found between wild trapped animals but not between laboratory born which had been bred on identical food. In all cases, the food choice seemed strongly influenced by previous learning: In the trapping habitat for the wild caught (the *Afl* were from beech forest, and the *Asy* from grassland), and through the food type used for breeding in the laboratory (cf. the differences found between wild trapped and laboratory bred, and between *Asy* fed on mash and *Asy* fed on pellets). However, when the laboratory born animals bred on the same food (mash) were compared, there were faint (beech) and strong (grass) differences between the two species in the same directions as found between the wild trapped, which may suggest the existence of some difference in innate dispositions that here had been overshadowed by identical learning (same feeding experience in the laboratory).

The hypothesis of HEINRICH (1951) that innate differences in food requirements and food preferences exist between the two species has not been much supported. Most investigations have been made on either species separately (HOLISOVA 1960, ASHBY 1967, WATTS 1968; DROZDZ 1966) and any apparent difference between the species may just have been due to differences as to the food available in the variety of habitats from which the mice of these investigations were derived. However HOLISOVA, no doubt referring to HEINRICH's suggestion, states that there were no indications of specific requirements in *Asy* for starchy over oily seeds. Furthermore, PFEIFFER &

NIETHAMMER (1972) making a direct comparison of the two species, with animals brought up in the laboratory, concluded that there seem to be no innate differences in food preference between them.

Future experiments concerning the possibility of innate differences between the two species, should be made with animals, which are brought up from weaning with both beech mast and grass spikes – and perhaps more types of natural food – available, since species specificity may also lie in a disposition to learn specific things. Furthermore, a too monotonous diet possibly has a deleterious effect on laboratory bred subjects' tendency to explore and try unfamiliar types of food such as the natural ones provided in the selection tests; this was indicated by the present results, where the wild trapped of both *Asy* and *Afl*, much more than the laboratory bred, tended to try (take and exploit) also the other, less prefered, type of seeds offered.

The fact that the *Asy* bred on pellets took more beech than the *Asy* bred on mash may be the consequence of a certain resemblance between pellets and beech spathes in size and shape. In the experiments of PFEIFFER & NIETHAMMER, the surprisingly great interest which the laboratory bred animals of both *Apodemus* species showed for larger tree seeds at their first exposure to this type of food, may have a similar explanation? There is, however, no information concerning the food used for breeding.

In the choice of foraging site, the two species showed differences independently of food, which correspond well with our previous results concerning habitat selection. It has been reported (CORKE 1970, HANSSON 1971), that *Apodemus* distribution may be affected by changes in the location of food supply. According to BERGSTEDT (1965) seasonal changes in *Asy* occurrence could be due to migration out of the woods in spring and back into woodlands in autumn. CORKE (1970) mentions that individuals of both species may perform such movements. On the other hand, as shown in the habitat selection and the feeding site selection experiments, *Afl* and *Asy* may show clear differences in distribution, when food supply is identical in woodland and grassland. Thus, other habitat parameters than food may be responsible as e. g. types of vegetation. It should be mentioned in this connection, that even during the occurrence of a peak number of *Afl* only few individuals of this species were trapped in grassland during the season of mature grass seeds, (HOFFMEYER & HANSSON 1974). The possible migration from grassland into woodland by *Asy* in the autumn may be affected by variations in *Afl* numbers in woodland. Thus, during an *Afl*-peak, almost no *Asy* were caught in beech forest during the beech crop season.

It was unexpected that *Asy* and *Afl* did not differ clearly in the tendency to feed up on the trees. *Afl* generally show a stronger inclination to climb (HOFFMEYER 1973), and in some experiments, which are being carried out at present, with trees that are taller and larger than in this experiment, *Afl* much more often than *Asy* are seen feeding on the sites up on the trees. The fact that in the free *Afl* have been observed high up in tall trees (BOROWSKI 1963), and that rather great numbers of *Afl* were caught in traps placed on trees (HOLISOVA 1969), suggest that this species can live on beech seeds before these fall to the ground. DROZDZ (1966) mentions that *Afl* willingly eat also the unripe seeds.

On the whole, the present results suggest that food factors may act by facilitating and/or enhancing segregation of the two *Apodemus* species. Selection of different habitats for other reasons than differences in specific food requirements implies that the two species encounter and experience different types of food. This acquisition of diverging food habits may be facilitated 1) by differences between the two species in ability to exploit the different types of food, which involves differences in effectiveness (e. g. here *Afl* penetrated more beech spathes than *Asy)* and 2) by differences between *Asy* and *Afl* in foraging range (vertical/horizontal distributions). Diverging food habits may

further enhance the habitat separation which primarily is caused by other factors (habitat preferences, competition).

A further way in which food could play a rôle in segregation of the two species, without being the direct cause of it, was suggested by some observations during the present experiments. Thus, *Asy* seemed to be more flexible in their feeding habits than *Afl:* 1) When tested over two consecutive nights, *Asy* from grassland showed a clear tendency to switch towards beech mast during the 2nd night, whereas *Afl* from beech forest did not show any opposite-direction-switch towards grass seeds; 2) the choices of laboratory born *Asy* bred on mash differed from those of *Asy* bred on pellets, indicating that this species can adapt to different types of food dependent on which is available. In contrast, some *Afl* bred in the laboratory and provided in tests with grass spikes only, did not even try to exploit this food and rather starved (average loss in weight per individual over 2 days = 8 g). Furthermore, if comparing the results of DROZDZ (1966) concerning *Afl* with those of HOLISOVA (1960) concerning *Asy*, the latter species seems to have a broader food spectrum. Unfortunately, however, the *Asy* of HOLISOVA's study were also taken from a greater diversity of habitats. A greater flexibility in *Asy* would allow them to live in a greater variety of habitats if forced to by various reasons (e. g. competition). However, more investigations are needed to state that such a difference in variability does in fact exist between the two species. Investigations should also be made concerning possible differences in the two species' ability to survive and/or reproduce successfully on either kind of seeds.

Summary

To investigate the possible rôle of food in the habitat segregation of two sibling species of mice *Apodemus sylvaticus (Asy)* and *A. flavicollis (Afl)*, they were compared 1) as to their choice between – and ways of treating – beech- and grass-seeds when these were provided in a simulated natural way, still sitting on the respective plants, and 2) as to their choice between foraging sites in an enclosure with woodland- /grassland-habitats. Wild trapped *Asy* and *Afl* from grassland and beech forest respectively, of an area in Sweden, where both species coexist, showed clear differences in selection and treating of the two types of food corresponding to the two habitats from which the mice were derived: *Asy* took more grass spikes than *Afl* and also exploited them more and *Afl* took more beech mast than *Asy* and were more effective in penetrating the spathes. Laboratory born *Asy* and *Afl*, which had been bred on identical food ("mash") only showed a tendency to differ in the same directions. The type of food used for laboratory breeding had an influence on the subsequent choice of natural seeds. *Asy* bred on pellets took relatively more beech than *Asy* bred on mash. Thus, even though both heredity and learning may play a rôle in the feeding of *Apodemus*, learning seems more important in causing differences between the two species. With regard to the selection of foraging site, *Asy* fed more in grassland and *Afl* more in woodland. It is suggested that food is not a primary cause of habitat segregation but that differences in habitat preference lead to the acquisition of diverging feeding habits, which may reinforce habitat segregation. Furthermore, food factors may facilitate segregation in the way of differences between the two species in their capacity to exploit seeds typical of either habitat, and through possible differences in flexibility (modifiability) of food preferences.

Zusammenfassung

Um zu untersuchen, welche Rolle Nahrungsfaktoren möglicherweise für die Biotop-Trennung der beiden nahe miteinander verwandten Waldmausarten *Apodemus flavicollis (Afl)* und *A. sylvaticus (Asy)* spielen, wurden sie verglichen mit Bezug auf: 1) Wahl zwischen und Behandlung von Bucheckern und Grassamen, wenn diese in natürlicher Weise – noch auf den Pflanzen sitzend – dargeboten wurden, und 2) Wahl zwischen Futterplätzen in den Wald- und Grasbiotopen eines Geheges. Wildgefangene *Asy* aus Grasland und *Afl* aus Buchenwald einer Gegend in Schweden, wo beide Arten nebeneinander leben, zeigten deutliche Unterschiede in Wahl und Behandlung der beiden Nahrungstypen entsprechend den bezüglichen Herkunftsbiotopen dieser Mäuse. *Asy* bissen mehr Grasähren von den aufrechtstehenden Stengeln ab und nützten sie auch besser aus, während *Afl* mehr Bucheckern im Gehäuse abpflückten und auch eine größere Anzahl der Bucheckerngehäuse durchnagten. Im Laboratorium geborene *Asy* und *Afl*, die mit gleichem Futter ("mash") aufgezogen worden waren, neigten dazu, sich in gleicher Weise wie die wildgefangenen zu unterscheiden. Das für die Haltung verwendete Futter beeinflußte die spätere Wahl der Tiere zwischen natürlichen Nahrungstypen: *Asy*, die mit Futterpillen aufgewachsen waren, nahmen mehr Bucheckern in Gehäusen als *Asy*, die an "mash" gewöhnt waren. Obwohl sowohl Vererbung als auch Lernen für die Nahrungswahl von *Apodemus* eine Rolle spielen, scheint jedoch hauptsächlich das Lernen die diesbezügliche Verschiedenheit zwischen den beiden Arten zu verantworten. Bei der Wahl des Futterplatzes fraßen die *Asy* mehr im Gras- und die *Afl* mehr im Waldbiotop. Es wird vermutet, daß die Nahrung nicht die Hauptursache der Trennung der Biotope der beiden *Apodemus*-Arten ist, sondern daß die Vorliebe für einen Biotop auch die für eine bestimmte Nahrung fördert, wodurch sich wiederum die Biotoptrennung verstärkt. Dazu könnte außerdem die artlich verschiedene Fähigkeit der Samenausnutzung der betreffenden Biotope und das artlich verschiedene Vermögen, Nahrungsvorliebe und -verhalten zu ändern, beitragen.

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INTRASPECIFIC INTERACTIONS IN WOOD MICE (APODEMUS SYLVATICUS L.) AND YELLOW-NECKED MICE (A. FLAVICOLLIS MELCH.)

A COMPARATIVE STUDY ON SPACING AND COHESIVE BEHAVIOR

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ABSTRACT

The occurrences of spacing/agonistic, i.e. distance-promoting behavior and cohesive/tolerant, i.e. contact-promoting behavior in Apodemus sylvaticus and A.flavicollis were compared. Adult animals, 158 A.sylvaticus and 140 A. flavicollis, trapped within the breeding season (May-August) from sympatric Southern Swedish populations were used. Interactions were observed in staged encounters between residents and strange intruders, and within established groups of 4 animals/group. Spacing behavior predominated in A. sylvaticus encounters whereas cohesive behavior predominated in A.flavicollis.

The differences in social tolerance could be understood from the different ecological adaptations of the two species in regard to habitat and feeding habits.

INTRODUCTION

The behavioral comparison reported in this paper concerns two closely related species of small rodents (family Muridae), which for a long time have puzzled European taxonomists and ecologists. The degree of morphological divergence varies geographically within their communal range in Europe (see e.g. Miller 1912, Ursin 1956 and Niethammer 1969). In my study area in Southern Sweden, adult individuals of the two species are however easily distinguished.

The ecological separation is even more unclear. Both species often occur in the same habitat, mixed deciduous forest (Bergstedt 1965, Montgomery 1980), although there is a tendency of segregation: A.sylvaticus inhabits also grassland, whereas A. flavicollis is mainly confined to woodland or forest. The degree of habitat overlap shows geographical, annual and seasonal variations (e.g. Löhrl 1938, Heinrich 1951, Felten 1955, Bergstedt 1965, Hamar et al. 1966, Corke 1974). Although there may be considerable overlap, they appear to prefer different vegetation types (Montgomery 1980),which is in accordance with my behavioral studies (Hoffmeyer 1973 and 1975, see I, appendix). I found the following features to be important: Trees for A. flavicollis, and ground cover for A. sylvaticus. Also, although both species are primarily granivorous (Hansson 1971, Pfeifer & Niethammer 1972), there are differences as to the seed types eaten predominantly (Heinrich 1951, Hoffmeyer 1976) probably because the two species are adapted to different vegetation types. In interspecific encounters in enclosure studies between animals from South Swedish populations, the larger A.flavicollis were generally dominant even though they usually did not show overt signs of aggression (Hoffmeyer 1973).

Differences in social behavior could have a genetic basis which determines, e.g., aggressive motivation (incl. reactivity to aggression-eliciting stimuli) and associated behavior patterns. Closely related animals often show similar behavior and differ only in frequencies of these patterns. Differences in social behavior between two species often reflect differences in ecology (see e.g. Crook 1965, Jarman 1974). Similarly, comparative studies on rodents showed "species differences" in social tolerance, reflecting differences in their typical social organisation in nature (Eisenberg 1962, 1967). Therefore, a comparison of the social behavior of A.sylvaticus and A.flavicollis should help to elucidate the ecological differences between them.

The majority of published information on social behavior concerns A.sylvaticus and very little on A.flavicollis. Comparative studies have been made by Montgomery (1978), and by Hoffmeyer (1973) and Hoffmeyer & Sales (1977). Montgomery (op.cit.) concluded that there was no difference in the two Apodemus species' social behavior, whereas I found clear differences during some preliminary observations. These differences were further examined and are reported in this paper. I have concentrated on such behaviors that contribute to the differences in distribution of individuals of the two species.

METHODS

ANIMALS AND TREATMENT

The test animals were adults from sympatric populations, trapped during summer (May-July).

Until the start of the experiment they were kept individually in standard plastic mouse cages (40 x 20 x 15 cm) with a wire mesh cover. Each cage had a shelter (23 x 8 x 7 cm). Wood shavings served as bedding, and cotton wool was provided as nesting material. The animals had water and standard mouse pellets (EWOS) ad libitum, supplemented with nuts and sometimes apples. They had a reversed day-night cycle of 16 hrs light and 8 hrs dark, and the observations were always made during the dark period, using red lights. According to previous studies (Hoffmeyer, unpubl.) A.sylvaticus generally started being active about two hours earlier than A.flavicollis, so the observation periods were planned accordingly. Tests with the two species were always run in parallel, in separate rooms. Experiments took place during July to November.

For testing, the animals were transferred to special observation cages (of varying dimensions, see Tabs 1 and 2) provided with an observation window and nest boxes of plexiglass. The back walls of the observation cages were painted light. The cages were provided with natural habitat cues: Tufts of grass, leaves on the floor and branches for climbing; food and water were provided ad lib. The observation cages were in separate rooms with the same light and temperature conditions.

Since these animals are very difficult to handle directly, live-traps (UGGLAN Special) were always used for transfers, in combination with polystyrene bags. Fur-clipping was used for individual identification, together with light-coloured ear-marks (HUMBROL). In all cases, conditions were the same for the two species.

In encounter tests I generally used the method resident x strange intruder. Wild trapped animals are very shy and need a period of familiarization with laboratory conditions. Elements of the animals' natural habitats were provided to facilitate the process. Each resident lived in the observation cage for 7-10 days before the tests.

TYPES OF EXPERIMENTS

In my comparative studies on Apodemus, I used some of the methods outlined by Eisenberg, whereby one can study social tolerance and arrive at some quantitatively based conclusions regarding species differences in sociability.

Interactions between conspecifics were observed in two types of experiments:

I) In staged encounters between a resident and a strange intruder males; and

II) In groups of 4 individuals per group, all male or heterosexual.

The method of encounter tests permits an assessment of the relative amounts of agonistic behavior displayed by a species.

I) Staged encounters between resident and strange intruder. The "resident" was an animal which had been living in the observation cage for a given length of time before the encounter was arranged by introducing a strange conspecific of similar size. Seventy-nine encounters of this kind were made with A.sylvaticus (involving 158 animals), and 70 encounters with A.flavicollis (involving 140 animals).

TABLE 1

Spacing or cohesive behavior in encounters between resident and strange intruder males (= home cage situation). The "residents" were males established in the observation cages. Each animal was used only in one test. The males paired were of similar size, but the intruder always a little smaller than the resident. Mean body weights A.fl.: 38.2 ± 8.9, A.sy.: 22.4 ± 4.4. A Mann-Whitney U-test, two-tailed (Siegel 1956), was applied for statistical testing of behavior differences based on the values from the single tests. xxx $p<0.001$; xx <0.01; x <0.05. ⊠ in all cases conditions were the same for the two species. For further information, see p.53-58.

Test Series No.	Test conditions: Time in captivity (days)	Test conditions: Size of observation cage (cm)	Number of animals involved (two per test): A.sylv.	Number of animals involved (two per test): A.flav.	Results: Type of behavior	Results: Occurrences mean values A.sylv.	Results: Occurrences mean values A.flav.	Results: (Mann-Whitney U-Test, two-tailed)
I-1	14	60 x 30 x 40	74	62	spacing	5.1	1.3	xx
					cohesive	1.2	1.8	NS
I-2	30	60 x 60 x 60	20	18	spacing	11.6	0.0	xxx
					cohesive	3.2	15.7	xxx
I-3	60	35 x 25 x 30	24	20	spacing	22.6	5.8	xx
					cohesive	0.7	5.8	xx
I-4	360	60 x 60 x 60	40	40	spacing	40.9	16.7	xx
					cohesive	1.1	7.7	xxx
		Total number of animals	158	140				

TABLE 2

Spacing or cohesive behavior observed within groups of 4 animals. The figures presented in the column Results are cumulated behavior occurrences from a fixed number of observations on each group.

Test series No.	Type of Groups (4 mice/group)	Test conditions		Number of animals involved (four per test)		Results		
		Time in captivity (days)	Type and size of obs. cage (cm)	A.sylv.	A.flav.	Type of behavior	Occurrences A.sylv.	A.flav.
II-1	all males	1-2 (grouped at start of test)	400 x 400 x 200 (indoor pen)	64	56	spacing	98	42
						cohesive	5	56
II-2	all males	360 (grouped 90 days before test)	200 x 300 x 140 (indoor pen)	16	16	spacing	34	30
						cohesive	4	82
II-3	heterosexual (2 males + 2 females)	60 (grouped just after capture)	120 x 60 x 60 (aluminium cage)	16	16	spacing	63	20

II) Encounters within groups of 4 animals.

The interactions between group mates were observed. Some of the groups were all-male groups (Series II-1 and II-2), others were heterosexual groups (Series II-3) with two males and two females per group. There were also some differences between the experimental series in the size of the observation cages, and in the animals' background. In Series II-1, the groups were observed immediately after the animals had been grouped, within one week of trapping. The animals in the Series II-2 and II-3 had all been in captivity for a longer period and had been living long time together (see Tab.2).

BEHAVIOR

Small rodents generally differ little in the very shape of social behavior patterns; so little that it has been possible to make a general ethogramme for small rodent research (Eisenberg and Eibl-Eibesfeldt, 1964). Differences rather appear in the frequencies of expression. The general lack of species-specific visible displays no doubt is correlated with the nocturnal habits of most of the rodents in cause.

Comparative studies on several small rodents favored the view stated by Eisenberg (1967) that there are species differences in social tolerance, which are the result of adaptation to different ecological niches.

The behavior was recorded by using the general terminology of van Oortmerssen (1972) adapted to the above mentioned glossary of terms used in rodent behavior studies by Eisenberg and Eibl-Eibesfeldt. It fits the behavior of Apodemus sufficiently for the present purpose, to compare the two species with regard to spacing and cohesive behavior.

The behavior elements grouped into these two main categories were:

Spacing behavior	1. Attack	Rushing and leaping at opponent with kicks and bites.
	2. Chase	Chasing a fleeing opponent. Looks like a prolonged attack
	3. Fight	The behavior shown by each of two animals, when locked together in violent wrestling behavior, the animals rolling over several times, so fast that separated behavior elements could not be discriminated properly (measured per series).
	4. Flee	Running away from opponent.

	1. Passing alongside	The behavior shown by two animals swiftly passing each other, touching each other lightly side toward side. (Behavior typical of A.flavicollis.) Gives impression of being a greeting act.
Cohesive behavior (contact promoting)	2. Crawl under	Pushing the head under the body of the partner and crawling under.
	3. Simultaneous groom	The two animals sitting closely together, side by side, licking and combing own fur.
	4. Social groom	Licking and combing the fur of the partner (mostly on the head or back) without signs of aggression (measured per series).

These recordings of behavior were made before the published work of Gurnell (1977) on behavioral elements displayed by A.sylvaticus; but the behavior elements used are quite similar. However, Gurnell classified "Crawl under" and "Social groom" as mutual acts of social investigation.

"Passing alongside" has not been described, but was typical of A.flavicollis' interactions.

Intermediate types of behavior, such as submissive upright, boxing, and withdrawal, were recorded but are not included in the present analysis.

Notes on the behavior were taken in short-hand in some of the test series, and with the aid of a multi-channel event-recorder (Miniscript) in others.

In addition to recording the behavior patterns chosen for comparison of interactions, estimates were also made, in the staged encounter tests (I-1), about the dominance outcome of each encounter. For this the following types of outcome were distinguished (see Tab. 3).

active dominance	$>_a$	One of the opponents was clearly dominant, and its dominance was manifested by aggressive behavior.
passive dominance	$>_p$	One of the opponents was dominant, but the dominance relations this time were manifested not by aggression from the dominant, but through the other one's avoidance behavior.
neutral	=	The two animals were mutually tolerant; they showed neither aggression nor avoidance, but often amicable behavior.

TABLE 3

Intraspecific dominance in A.sylvaticus and A.flavicollis. In each case recorded dominance was based on several recordings. Social interactions were classified into three main types: $>_a$: active dominance; $>_p$: passive dominance; = : mutually tolerant or amicable (see p.58)
a) In test series I-1; b) In test series II-1.

Species	Number of staged encounters between conspecific males	Social dominance $>_a$	$>_p$	=
A.sylvaticus	37	28	6	3
A.flavicollis	31	13	4	14
	Number of observed encounters between conspecific males in groups			
A.sylvaticus	156	98	53	5
A.flavicollis	137	42	39	56

RESULTS

A.sylvaticus generally showed more spacing behavior than A.flavicollis, whereas A.flavicollis generally showed more cohesive behavior than A.sylvaticus. This trend was found both in the staged encounters, between residents and strange intruders (Tab. 1), and within the groups of 4 animals (Tab. 2). The difference in spacing behavior appeared most clearly in the staged encounters, whereas the difference in cohesive behavior was about the same in the two types of experiments (Tab. 1 and Tab. 2).

In addition to the two main categories of behavior presented in the tables, the frequencies of "social investigation", i.e. the animals sniffing each other, were recorded in one of the staged-encounter test series (I-4). The mean frequency of social investigation for A.sylvaticus was 1.1 per encounter and for A. flavicollis 6.3. The difference was statistically significant

($p<0.01$, Mann-Whitney U-test). The frequencies of agonistic behavior increased in tests where the animals had been held in captivity a long time and the residents were well established (Tab. 1). The sizes of the observation cages did not affect the general trend in the results.

In the groups the same trends appeared: A.sylvaticus showed more spacing than cohesive behavior, while it was the opposite with A.flavicollis ($p' < 0,05$, Fischer-test, two-tailed, Siegel 1956). In Series II-3, I only recorded spacing behavior. In the 12 half-hour observations on each species spacing behavior occurred during 11 of the 12 observations on A.sylvaticus, but only in 3 of 12 observations on A.flavicollis.

CONCLUSION AND DISCUSSION

SOCIAL BEHAVIOR IN A.SYLVATICUS AND A.FLAVICOLLIS

In the present experiments, violent agonistic encounters (attacks, chases, fights) as well as mutual flight reactions occurred more often in A.sylvaticus than in A.flavicollis. Thus, spacing behavior was more common in A.sylvaticus. Conversely, contact promoting behavior was more common in A.flavicollis, as well as encounters in which the participants were mutually tolerant, without signs of aggression. These results confirm previous observations (Hoffmeyer 1973).

Male aggression in A.sylvaticus has been discussed by several authors. Effects of it are reported from the field (Brown 1966, Flowerdew 1974, Montgomery 1980). However, in the laboratory Gurnell (1977) found a low frequency of aggressive acts between males in a neutral cage. In a later study, he observed space-associated intolerance at the start of the breeding season, and also a strong tendency for mutual avoidance (Gurnell 1978). Adult males can suppress younger ones during the breeding period (Flowerdew 1974). Aggression between adult males was observed also during the non-breeding season in a stable group in a large indoor pen (Bovet 1972); and it has been reported from other laboratory groups in smaller cages (Richard-Yris 1979). Some authors have suggested that a social hierarchy may exist; Brown (1966) from direct observations in the wild and Richard-Yris (op. cit.) from laboratory observations. However, Gurnell (1972) did not recognize such hierarchies in stable colonies.

The social behavior of A.flavicollis is much less known, possibly because this animal is more difficult to handle. Some authors have reported that A.flavicollis show aggression toward the bank vole (C.glareolus) when they occur in the same habitat (Andrzejewski & Olszewski 1963). However, in my studies A.flavicollis rarely behaved aggressively toward A.sylvaticus; interactions between these two species were generally characterized by A.sylvaticus avoidance (Hoffmeyer 1973).

Zimmermann (1955) reported that captive A.flavicollis individuals frequently displayed "acts of mutual tenderness" ("gegenseitige Zärtlichkeiten"), and also "worked together" when gnawing a hole through the cage wall. Andrzejewski & Olszewski (op.cit.) found that "tolerant meetings" took place between A.flavicollis in 67 % of all meetings observed between individuals of this species in their natural habitat. Also, it was observed in outdoor enclosure conditions that all the individuals of A.flavicollis in winter occupied only one of the nest boxes offered to them (Olszewski unpubl., see Fedyk 1971). But in all these cases the individuals were probably familiar with each other.

Montgomery (1978) who compared animals from sympatric populations in England, concluded that there was no difference concerning intraspecific social behavior of A.sylvaticus and A.flavicollis, and no indication that A.sylvaticus encounters involved more agonistic acts than A.flavicollis encounters. However, this divergent result was probably due to the test conditions. In Montgomery´s experiments, encounters were arranged in a "neutral arena", i.e. in a cage which was new to both participants. Under such conditions, the animals have a strong tendency to explore the area and this may be the reason why agonistic acts were generally few. The same may apply to the results of Gurnell (1977). My first experiments on interaction in the two Apodemus species (Hoffmeyer 1973) were also performed in an arena where the animals had never been before. However, in contrast to that of Montgomery and Gurnell, my arena was provided with natural habitat cues, which probably reduced the novelty effect. In the present experiments, the effect of a new cage was further reduced as one of the opponents was resident in the observation cage, and the animals were kept in captivity for a longer time before being tested.

The fact that A.flavicollis are able to live in groups without serious fighting between males suggested that they use other

behavioral mechanisms for control. Ultrasound emissions were very frequent in close contact between individual males (Sales & Hoffmeyer 1977), while in A.sylvaticus they were mostly emitted by single males moving about alone. Also visual signals may be involved in communication between A.flavicollis individuals. Their bright white ventrum displayed in combination with frequent "upright" postures during social interaction may have this function, as it gives a flashing effect. Finally, but not least, is scent communication likely to be of great importance in this species. (Stoddart, 1974).
Differences as to the time young stay together with parent(s) and sibs cause differences in early social learning.

In A.sylvaticus the young of the first litter usually disperse early, while in A.flavicollis the young of the first litter often occupy the nest during the rearing of the second litter. This clearly must enhance the divergence in spacing vs social tolerance between the two species.

SEASONAL VARIATIONS

There are seasonal changes in behavioral interaction observed e.g. in American deermice, Peromyscus maniculatus (Sadleir 1965, Healey 1967). A.sylvaticus showed an increase in aggression from winter to spring and a peak in agonistic behavior of home caged males occurred in May (Gurnell 1978). Intolerance by resident adult males might be important during summer too and might lower the survival of young and immigrants (Flowerdew 1974).

Seasonal fluctuations in agonistic behavior may not be synchronous in the two species: The distributions of food items eaten by the two Apodemus species appear more divergent during summer than during autumn and winter (see e.g. Corke 1974, Hoffmeyer 1976), and the reproductive season of A.flavicollis starts before that of A.sylvaticus in areas with both species (Jüdes 1979, Montgomery 1980b).

Differences in social behavior between A.sylvaticus and A.flavicollis found during spring and summer might be less pronounced in autumn. A.sylvaticus may show less spacing behavior in autumn, when the distribution of their food becomes more similar to that of A.flavicollis. Gurnell

(1978) reported less space-associated aggression/intolerance in A.sylvaticus during autumn and winter, and Bovet (1972) found fluctuations in aggressiveness in A.sylvaticus also throughout winter. In A.flavicollis there might be more aggression during winter; the formation of dominance hierarchies should be particularly important in relation to the vital but limited food stores, and the reproductive season of this species is reported to start as early as January (Montgomery 1980 b). A peak of aggression in A.flavicollis was also observed in winter (Hoffmeyer unpubl.).

ECOLOGICAL CAUSES OF DIFFERENCES IN SOCIAL BEHAVIOR BETWEEN A.SYLVATICUS AND A.FLAVICOLLIS

The differences in social behavior presumably reflect differences in ecology. Information may be obtained by the comparative method: Similarity in social behavior can exist between distantly related species, which live under similar ecological conditions (convergent evolution), while there are differences in social behavior between closely related species with different ecology (divergent evolution) (see e.g. Crook 1965).

According to socio-ecological studies on other species, differences in social behavior of the kind found here may be related to differences in food distribution, habitat type, activity range, and predation risks (Crook 1965, Horn 1968, Jarman 1974, Kruuk 1975). These ecological variables interact with species parameters (morphological and physiological characteristics) which determine the animal's mobility, susceptibility to predation, mating and rearing systems. All these factors contribute to shape a given type of social system (Crook et al. 1976).

Type of habitat (structural features of vegetation). To explain the differences in social behavior between the two Apodemus species, the following characteristics in the vegetation are considered: First, being "open" or "closed"; and second, providing a more or less arboreal living space.

Large aggregations of animals (e.g. herds of ungulates or flocks of birds) are in general associated with "open" habitat types; open field in contrast to forest. However, for small rodents conditions could be just opposite: Dense ground vegetation of many open field habitats (e.g. grassland) representing the "closed" habitat type in comparison with a forest floor, which often has

little or no ground vegetation. The reasons for animals being gregarious in "open" habitats generally are: 1) the advantages of grouping as a defence strategy when no shelter is available (e.g. Crook et al. op.cit.) and 2) the "open" habitat giving less hindrance to group cohesion.

The other aspect related to structural features of habitat, which is supposed to have influenced the shaping of Apodemus social behavior is the access to an arboreal living space. It appears that arboreal species often are less aggressive than ground living ones (e.g. Ewer 1971). Tree-living species might face less competition for space, and violent attacks and chases may be less practicable on trees than on the ground.

Predation. The divergence in social behavior between the Apodemus species may also be related to predation. Grouping is one defence strategy (Hamilton 1971). For small rodents, which live in habitats without cover at ground level to provide nearby refuges it must be important to have a defence system, that augments the chances of detecting predators early, and collective vigilance is one such possibility. Furthermore, the cost of being in group by being more easily observed by predators may be outweighed if each of the individuals keeping together is itself well equipped for predator avoidance.

With reference to Apodemus, A.flavicollis individuals were more often observed moving about together than were A.sylvaticus individuals, and A.flavicollis have more efficient defence devices than A.sylvaticus (Hoffmeyer 1973, App.). These observations provide further support for the hypothesis that A.flavicollis are adapted to habitats with less vegetation cover at ground level in contrast to Apodemus sylvaticus for which spacing out should be a better defence strategy.

Food. The distribution of food in space and time is one of the strongest determinants of social systems (Crook 1965, Horn 1968, Jarman 1974, Crook et al. 1976). In general, defendability is likely to correlate with evenly dispersed resources, whereas spatially and temporally heterogeneous supplies are unlikely to be defended (cf. Crook et al. op.cit.). Furthermore, patchily distributed resources often favor group formation: Large clumps of food with a distribution unpredictable in space and time are better exploited by several animals together, because of the

advantages for detection, mutual information (e.g. local enhancement) and collective defence. However, if food is patchily distributed in quantities so small that only single animals can profitably feed on them, group cohesion is adversely affected because the individuals may not be able to travel together in permanent close-knit groups. They have to separate during feeding, as found by Jarman (1974) for antelope.

My experiments on food in the two Apodemus species (Hoffmeyer 1976) concerned two seed types representing two opposite extremes: Large tree seeds of deciduous forest (beech mast) and small grass seeds (Dactylus) of grassland. The results indicated how food may have contributed to the differences in social behavior of the two Apodemus species: The small seed types (grass seeds) often used by A.sylvaticus represent evenly dispersed food resource in grassland habitats occurring in quantities too small to be profitably exploited by group foraging. This is in accordance with the predominance of spacing behavior in adult A.sylvaticus during summer, which assures the spacing of individuals in relation to the evenly distributed food. Conversely, the larger energyrich tree seeds, essential for A.flavicollis, occur as clumped food resources, both spatially (on single trees, or somewhere at their base) and temporally (in late summer, autumn, start of winter); furthermore, their occurrence is fairly unpredictable with great variation in abundance from year to year. This kind of food distribution may explain why cohesive (contact-promoting) behavior predominated in A.flavicollis, favoring formation of the large aggregations of individuals in some summers (observed by Montgomery 1980; and myself).

Since availability of clumps of beech mast or acorn is limited in time, a need for persistent hoarding for short intervals may have further promoted the gregarious tendencies in A.flavicollis, which depend more on this food type than do A.sylvaticus (Hansson 1971, Hoffmeyer 1976) a.o. because the larger A.flavicollis require a greater amount of concentrated food per day. Behavioral mechanisms have evolved assuring the collection and hoarding of the seeds as efficiently and quickly as possible. Keeping together for defense (mutual vigilance) while foraging on uncovered ground below a big tree may have facilitated cooperation, which seems a profitable solution if the cost of sharing the food is below the benefit for the individuals. A.flavicollis individuals, which stay together, could be related and so

collaborative behavior could have evolved by kin selection.

Both Apodemus species have the possibility to switch to alternative food to supplement the seeds; food choice results (Hoffmeyer unpubl.) indicated a greater food flexibility in A.sylvaticus than in A.flavicollis. A.sylvaticus individuals sometimes change to larger tree seeds in autumn. Still the cost of handling may be relatively higher for A.sylvaticus while the beech nuts are still on the trees or in their spathes (Hoffmeyer 1976), and this gives A.flavicollis a competitive advantage. In years with high A.flavicollis numbers A.sylvaticus must to a greater extent keep to other available food within their preferred vegetation types, including animal food, fungi, roots, and green food. The distribution of these items favors solitary hunting with the spacing of individuals again.

From the given review of general correlations between social behavior and ecological parameters, I conclude that the differences in intraspecific interaction I have found between Apodemus sylvaticus and A.flavicollis give support to my earlier results concerning ecological differences between the two species,(Hoffmeyer 1973,1975 and 1976).

Intraspecific variations might be found if the ecological correlates of given social types change within different parts of a species' range (see Kruuk 1975), or if there are important fluctuations in numbers. This aspect is at present being studied in Northern (fluctuating) and Southern (stable) Scandinavian populations of the bank vole.

SOCIAL BEHAVIOR AND REGULATION OF APODEMUS SYLVATICUS AND A.FLAVICOLLIS POPULATIONS

The difference in social behavior found within the two Apodemus species may help to explain field data on their population structure and dynamics

Social behavior, particularly male aggression, probably is important for the regulation of A.sylvaticus numbers (Watts 1969 Flowerdew 1974, 1978 and Gurnell 1978). The observed spacing behavior in A.sylvaticus (during spring and summer) may explain the observed low and stable numbers of males during much of the breeding season (Montgomery 1980).

A regulating effect on numbers by male aggression may operate through dispersal or through suppression of subordinate males. An increase in space-associated intolerance has been observed at the beginning of the breeding season (Gurnell 1978). This might cause young males born during spring and summer to disperse. Evidence from field studies suggests that adult males affect survival of juveniles (Flowerdew 1974). As mentioned above (p.60) a social hierarchy observed in home range studies (Brown 1966, and also in the laboratory in unisexual or mixed captive groups (Bovet 1972, Richard-Yris 1979) was clearly revealed when a stranger was added. Accordingly the suppression of subordinates reported by Flowerdew (1974) should be enhanced during periods with maximal interaction between strangers (i.e. at high population A.sylvaticus numbers, and/or when dispersers meet with resident animals).

Conversely, the role of aggression in regulation of A.flavicollis populations has not been studied. Mutual tolerance as mostly observed in the present encounter tests with A.flavicollis should facilitate aggregation. This could explain the marked concentrations of A.flavicollis individuals during summer, reported by Montgomery (1980) and which contrasted with the generally low and stable A.sylvaticus numbers during the same period. A.flavicollis often show important fluctuations in numbers connected with beech-mast abundance (Hansson 1971, Hoffmeyer and Hansson 1974); however, 3-year cycles in A.flavicollis numbers in Germany are reported to be independent of acorn-crops (Wendland 1975, 1981). Regulation by intrinsic factors (social regulation) was suggested.

Sociality often implies cooperation between individuals. Social thermoregulation seems to be important in A.flavicollis (Fedyk 1971), to reduce energy expenditure during the cold season. However, thermoregulation may as little as mutual vigilance for predator avoidance be regarded as cooperation in the true sense.

In the given account of the possible causes of differences in social behavior I focused on the three main ecological factors responsible for variations in the social organization of animals. According to theories of modern evolutionary thinking (Hamilton 1963, Maynard-Smith 1977), relatedness between individuals is an important determinant of sociality because being together with and

helping relatives, the individual may increase its own inclusive fitness.

Because mortality (predation) is generally high in the small rodents, the aspect that behavior of an individual may increase the chances of survival of its genes in relatives, should be particularly important in this taxonomic group.

Therefore studies on mating systems and dispersal are suggested for future research on the two Apodemus species as these factors determine degrees of relatedness among individuals and thereby their social behavior.

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SCENT MARKING, SOCIAL STATUS AND MATING COMPETITION IN BANK VOLES

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ABSTRACT

Urine marking of dominant and subordinate male bank voles was examined by exposing the males to a conspecific male or female in caged experiments. The test animals were from laboratory groups with stable social hierarchies. The test period was kept short to allow identification of separate urinations, which were collected on filter paper and later visualized by UV-light. Dominant males marked more frequently and their marking pattern was characterized by many trace- and small-spot-urinations. Subordinate males rarely trace--marked. The low marking rate in low ranking males was not increased during short-term encounters with a female; but long-term cohabitation caused an increase in the marking frequency of such males. Males with the greatest success in mating tests with primiparous females showed higher urine marking frequencies than the less successful ones.

The results are discussed with regard to biological aspects. The difference in marking implies that subordinate males should be less efficient in male x male competition/territorial defence. Furthermore, the difference may contribute to female mate choice in favor of the dominant males.

INTRODUCTION

In many small rodents, the urine of adult males plays an important role in chemical olfactory communication, causing several behavioral and physiological responses (see reviews by Stoddart 1974, Bronson 1976, Christiansen 1976, Brown 1979). Most of the research has been done on laboratory mice and rats, with an emphasis on questions related to small rodent reproduction, including intermale competition: aggression, aversion and social status.

The urine of male laboratory mice (Mus musculus) contains odorous factors which attract conspecific females (Scott & Pfaff 1970), elicit aggression in conspecific males (Mackintosh & Grant 1966, Mugford & Nowell 1970), cause aversion in males (Jones & Nowell 1973), or signal the social status of the donor to both males and females (Jones & Nowell 1974). These effects of male urine have been attributed to secretions from the preputial glands (Bronson & Caroom 1971, Brown & Williams 1972).

In male bank voles the increase in size of preputial glands is associated with sexual maturation (Christiansen, Wiger & Eilertsen 1978) and the difference in size of these glands between dominant and subordinate males (Gustafsson, Andersson & Meurling 1980) suggested a behavioral role of the preputial gland sebum.

The pattern of urine-marking by male mice was described by Desjardins, Maruniak & Bronson (1973) with a method of visualizing by UV-light. For the bank vole, urine marking was first de-

scribed by Johnson (1975), who reported differences between races and between sexes. Christiansen (1980) found that urine trace marking, characteristic of male bank voles, was dependent on season and sexual maturation and he suggested that the fine trace urinations serve the purpose of depositing preputial gland sebum, with the functions of sexually attractive or socially repulsant signals, or both.

Male Clethrionomys may form stable dominance hierarchies, with one male being dominant over other males throughout the breeding season. This was found both in the field (Viitala 1977 with Clethrionomys rufocanus and Sörensen 1982 with C.glareolus) and in the laboratory (Sörensen 1981 with C.glareolus).

The present investigation aimed 1) to examine the urine marking of male Clethrionomys glareolus in relation to their social status and exposed to a strange male intruder, and in other tests to a female; 2) to examine the effect of short- and long-term presence of a female on subordinate males' marking, and 3) to examine mating success of individual males in relation to their marking frequency.

ANIMALS AND TREATMENT (GENERAL)

Adult male and female Clethrionomys glareolus from a laboratory colony (Gustafsson, Andersson & Westlin 1980) were used in the experiments. The animals were kept in standard plastic mouse cages (40 x 20 x 15 cm) with a wire mesh cover on a photoperiod of 18 hrs light/6 hrs dark and a temperature of 22±5°C. Wood shavings were used as bedding. The animals were allowed water and a commercial mouse food ad libitum, supplemented once a week with standard rabbit- and guinea pig-pellets.

After weaning, at 18 days of age, they were placed in single-sex groups of 5, all housed in the same room. The males were furclipped for individual identification. For at least 1 1/2 - 2 months before the start of the experiments, the dominance relations within each male group were assessed through weekly observations. To determine social dominance in groups of bank voles, I used the intruder method modified after Sörensen 1981. Dominance is positively correlated to specific types of behavior (flank scratching with hindleg, displacement digging, attack); subordinance to escape and submissive upright posture (Sörensen, op.cit.).

EXPERIMENTS

EXPERIMENT 1

Marking by resident males exposed to a strange male intruder.

Differences in urinary marking between male bank voles may influence the effect these males exert on conspecific males (see VI and supplement). The aim of this experiment was to compare the marking of dominant and subordinate males when in presence of a strange male intruder.

Procedure. Urinary markings were obtained as described by Desjardins et al. (1973) in their study on house mice, with some modifications:

a) the experimental males of clear social status (dominant or low ranking subordinate) were derived from male groups in which the dominance hierarchy had been stable for at least one month, according to weekly observations (see p.73).

b) the males were tested in their home cage in order to increase reactions to the strange intruder, and

c) the test duration was short (10 min of activity) in order to obtain each urination as distinct as possible and with a minimum of overlap; to avoid too much elimination urine (= non-marking urinations); and to prevent the animals from chewing the paper.

Markings by the resident experimental males were obtained on filter paper, fixed on a plexiglass plate, which covered the cage bottom. In the middle of the plate a small wire-net box (a live trap UGGLAN SPECIAL) was placed containing the stimulus animal ("intruder"; Fig. 1).

The males were tested individually. Each one was isolated in its home cage, 30 minutes before a test, by removing the other males of the group. At the same time the standard cage lid of wire-mesh was replaced by one of perforated plexiglass to prevent the test animal from climbing on it during the test period. The stimulus animal (intruder) was put into the small wire-net box 10 minutes before the test. This box was then placed on the marking plate just before being silently introduced into the cage of the test male, who meanwhile had hid in the woodshavings at the other end. A test period started, when the resident male first moved up on the plate, and was ended when it had been active on the plate for 10 minutes.

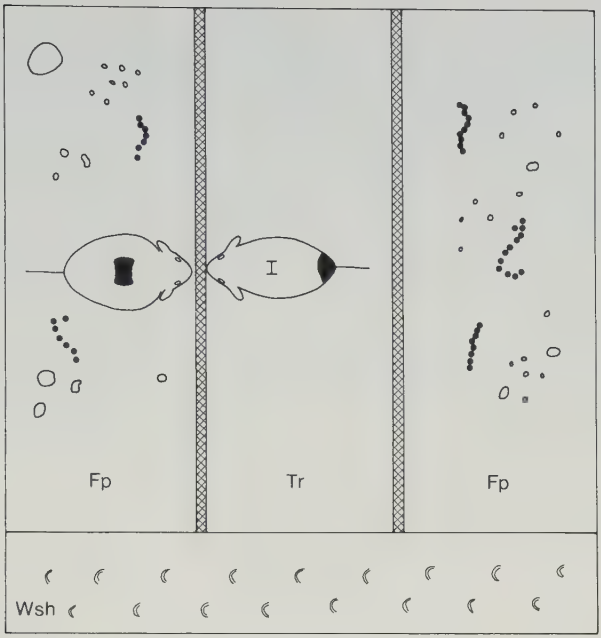

Fig. 1. Set-up for obtaining the urine marking of resident male bank voles when confronted with a conspecific 'intruder', (view from above). The intruder (I) is placed in a trap (Tr) in the home cage of the test animal, which marks on filter paper (Fp) attached to a plexiglass plate. Wsh = wood shavings left at one end of the cage. The two animals can sniff each other through the walls of the trap made of wirenet (mesh width 5 mm).

The urinations of each male were visualized by means of ultraviolet light. The contours of urinations were drawn with a fine pencil. The following urinations, described by Johnson (1975) and illustrated in Fig 1, were distinguished: a) Fine traces (or trace urinations) of a series of at least five 1-1.5 mm Ø spots. Sometimes these spots had fused, but they clearly formed a trail. Christiansen (1980) used "total length" of the fine traces as a measure, but I chose to count each trace urination separately because this expresses the frequency of marking by the animal. b) Small spots which were distinct, scattered and about 2-5 mm Ø.

The distinction between the above urination types may be useful for describing the marking pattern of different males. Here attention was mainly paid to the occurrence of the so-called fine

traces (Johnson op.cit.) because of their connection with deposition of preputial gland secretion suggested by Christiansen (1980) and later demonstrated by Brinck & Hoffmeyer 1983.

Twenty-four males (12 dominants and 12 subordinates) were tested with strange male intruders, a subordinate male and a dominant male from other groups, presented in random sequence with intervals of 4-7 days between the tests. Eight additional males (4 dominants and 4 subordinates) were only tested with a subordinate male intruder.

In mice, urine marking by intact non-subordinated males is largely a response to novelty, physical or biological (see reviews by Bronson 1976 and 1979). Possible variation in relation to the novelty factor was reduced by always using strange intruders and rather long intervals between the tests.

TABLE 1, (Exp.1)

Urine marking by dominant and subordinate male bank voles in the presence of a strange male intruder. Mean number of urinations per 10 min active time. The males were tested individually in their home cage, and urinations were collected on filter paper (total area 265 x 130 mm). The intruder was confined in a live-trap (UGGLAN-Special) of wire-mesh (mesh width 5 mm) (Fig.1). N = number of males tested, D = dominant males, S = subordinate males.

Test situation	Test animals	N	Total urinations (means ± SE)	P
with dominant male intruder	D	12	57.2 (±9.4)	<0.002
	S	12	4.1 (±1.7)	
with subordinate male intruder	D	16	67.6 (±8.4)	<0.002
	S	16	5.8 (±1.8)	

P = probability; D > S according to Mann-Whitney U test (two-tailed).

Results. The marking frequency and pattern of male bank voles is correlated with social status.

Dominant males marked significantly more than subordinates with both types of male intruders (Tab. 1). Trace urinations were also more frequently produced by dominant males (Tab. 2).

TABLE 2, (Exp.1)

Trace marking by dominant and subordinate male bank voles in the presence of a strange male intruder. For further information see Table 1. N = number of males tested. D = dominant males, S = subordinate males.

Test situation	Test animals	N	no.of males which trace marked	P	no. of traces (means ± SE)	P
with dominant male intruder	D	12	11	0.01[a]	7.8 (±2.7)	<0.02[c]
	S	12	0		0.0	
with subordinate male intruder	D	16	14	0.001[b]	16.9 (±3.4)	<0.002[c]
	S	16	2		0.1 (±0.1)	

P = probability according to [a]Fisher test, [b] X^2-test, [c]Mann-Whitney U test (two-tailed).

Some variation in the general marking pattern appeared. A few dominant males seemed to be less able than most others to control the quantities of urine voided with each marking; they showed a pattern with trails or spots somewhat larger than normal for dominants. Conversely, some of the subordinates, despite low marking rates, appeared able to control the urine quantities voided, making very small spot-urinations. The marking frequency of a subordinate male was sometimes a little higher (statistically insignificant) when it was confronted with another subordinate, than when it was confronted with a dominant; these variations were probably due to the relative rank relations between the test- and stimulus-males.

EXPERIMENT 2

Marking by dominant and subordinate males in the presence of females.

Differences in urinary marking between male bank voles might have an influence on female mate choice (Hoffmeyer 1982, Brinck & Hoffmeyer 1983). Also the presence of a female sometimes stimulated the marking activity of subordinate male bank voles, i.e. she appeared to counteract the inhibitory effects of subordination on marking (Hoffmeyer 1981).

The aim of the present experiments was to compare the marking of dominant and subordinate males when in contact with females.

Procedure. The set-up used was somewhat different from that of Experiment 1. Since female bank voles normally will start to reproduce only when settled in a home area (Viitala 1977), the test conditions here simulated a situation in which resident females are simultaneously visited by two males with different social status.

The stimulus females were in their home cage, which opened into two separate compartments of the same size. Each of these was lined with clean filter paper on the floor for male markings, and had one side of wire-net (mesh width 5 mm) toward the female cage. At the start of a test, a dominant male was placed in one compartment and a subordinate male in the other. The two males were group mates with dominance relations as in Experiment 1.

Sixteen pairs of males from 16 different male groups (not those of Experiment 1) were used.

Results. The urine marking scores of the dominant males were significantly higher than those of the subordinate in 14 of the 16 pairs tested (Tab. 3).

TABLE 3, (Exp.2)

Urine marking by dominant and subordinate male bank voles when both (simultaneously) in presence of females. 16 pairs of dominant-subordinate male group mates, derived from different groups, were tested (other males than those of Exp.1). Marking scores of dominant vs subordinate were compared within each pair. N = number of pairs tested, D = dominant males, S = subordinate males. (The defecation scores are presented too because they showed a remarkably opposite trend!)

	N	Number of dominant-subordinate male pairs where		P
		D marked more than S	S marked more than D	
Urine marking	16	14	2	0.002
(Defecation	16	1	14	0.002)

P = probability according to Binomial test ($p = q = 1/2$).

EXPERIMENT 3

Effects of females on the marking activity of subordinate males.

The differences in male marking would be of little importance with respect to mate choice, if the depression of marking by subordination could be momentarily relieved when subordinate males encounter a female in the absence of the dominant male. In tests similar to those of Experiment 1, subordinate males sometimes increased their marking activity when tested alone with a female (Hoffmeyer 1981). However, this could be due to the experimental procedure, where the female stimulus animal was always presented last in a sequence of tests.

1. To investigate whether short-term encounters with a female really do cause an increase in the marking activity of subordinate males, a series of tests was made in which other subordinate males were presented with male and female intruders in random order at 1 day intervals. Like in Experiment 1 the male intruders were strange subordinates derived from other male groups. Twentyfour long-time subordinate males were tested.

Of the 24 test males, 10 marked more in the presence of a female, 12 marked more with the male intruder, and two showed no difference. Thus, there was no tendency that long-time suppressed male bank voles increased their marking activity in short-term encounters with females.

2. To investigate the effects of long-term presence of females, ten other subordinate experimental males were tested for urine marking in the presence of females, before and after a 21 day period of cohabitation with a female (not the female used as stimulus animal in the tests). Ten additional subordinates, serving as controls, were only separated from the dominant male for 21 days, and tested before and after like the experimentals.

When the markings were compared, the experimental males after long-term presence of a female had a substantial increase in marking (mean increase in number of traces 10.2), and nine of the ten males marked more after they had been with the female than before. The control males (which had only been separated from the dominant) did not show a similar strong increase; only six of ten marked more after separation than before, and the mean increase for these was only 5.0 traces.

EXPERIMENT 4

Marking by males with different mating success, in tests with primiparous females.

Earlier experiments suggested that urine marking activity of males might influence their mating success (Hoffmeyer 1982, Brinck & Hoffmeyer 1983). The aim of the present experiment was to compare the marking activity of male bank voles, which were differentially successful in mating tests with primiparous females.

Procedure. The males were of similar age and background. They were kept singly, and their social status was unknown. They were paired with an equal number of females - one per day - and the number of achieved matings (decided by the presence of vaginal plugs) were counted. Subsequently, the males were tested for urinary marking in presence of a female intruder, using the method described in Experiment 1.

Results. Generally, marking frequency was higher in males having achieved a high number of matings than for males less successful in mating (Tab.4).

TABLE 4, (Exp.4)

Urine marking by male bank voles differentially successful i mating tests with primiparous females. The males were of similar age and background, but they were kept individually and their social status was not known. Procedure as in Exp.1 (see Tab.1), but a female was used as intruder.

	N	Number of males with urine marking frequency		P	Number of traces	P
		high (>20 marks)	low (<10 marks)		(means ± SE)	
A: males with high mating success (> 6 females)	9	7	2		18.2(±4.9)	
				0.05[a]		0.02[b]
B: males with low mating success (< 3 females)	8	2	6		1.1(±0.6)	

P = probability A > B according to [a]Fisher test, [b]Mann-Whitney U-test (two-tailed)

DISCUSSION

URINE MARKING IN DOMINANT AND SUBORDINATE MALE BANK VOLES

The frequency and pattern of male bank vole urinations were found to depend on social rank: Dominant males produced more trace and small spot urinations. The results are similar to those obtained by Desjardins et al. (1973) with house mice. The occurrence of trace urinations is of special interest because Christiansen (1980) found that only sexually mature males would trace-mark. However, in my experiments all the males were sexually mature, so trace-marking depended on social rank since it was shown mainly by dominant males and rarely by low ranking ones. Also the small-spot urinations were more frequent in dominant males. According to Johnson (1975): "both types of urination satisfy a criterion by Kleiman (1966) to distinguish markings from normal acts of urine elimination; they constitute modifications in the manner and frequency with which elimination occurs, the proposed function being to save urine for marking purposes and assure its spread over a larger area. Both types give evidence of the animal's ability to control the quantities of urine voided at each instance, and such control may well be connected with the release of specific glandular substances." The high marking frequency of dominant males is related to the much larger size of their preputial glands; and the high frequency of traces supports the suggestion (Christiansen op.cit.) that this type of urination is involved in the deposition of preputial gland secretion. Recently, the results of chemical analyses provided evidence for this hypothesis (Brinck & Hoffmeyer 1983).

The difference between dominant and subordinate males in trace marking appears significant in scent communication because the bank voles respond more strongly to marking, i.e. to trace urine than to metabolic urine, which constitutes the larger spots (Brinck & Hoffmeyer 1983).

Urine marking behavior in mice is influenced by hormones and experience (reviews by Bronson 1976 and 1979). In males it needs testosterone support (Maruniak, Desjardins & Bronson 1977) and the same appears to be the case with bank voles (Christiansen 1980). Within stable dominance hierarchies dominant males generally have higher androgen levels than subordinates (McKinney & Desjardins 1973), and the subordinate male bank voles tested here had a long-term coexistence with the dominant of their group. The androgen levels in the subordinates were lower than in the domi-

nants as indicated by smaller preputial glands in the subordinates; the size of male preputial glands is androgen-dependent (Brown & Williams 1972, Jannet 1978).

However, inhibition of marking may occur as a rapid response to experiencing defeat in agonistic encounters (Maruniak et al. op.cit.). It is possible therefore that the low marking activity of the subordinate male bank voles here was also due to frequent agonistic interactions with the dominant of their group. These interactions were often elicited by the strange male which was introduced to assess the group hierarchy (see p.73). Under natural conditions, one may expect a similar situation with frequent agonistic interactions at high population densities, and during dispersal of young. Pilot studies in this laboratory have indicated that early exposure of young male bank voles to violent aggression from adults is particularly efficient in causing persistent inhibition of marking. This is interesting because at high population densities young voles are likely to be exposed to aggression earlier in life and more frequently. The existence of a "critical period" for maximal suppression of marking should be a topic for future research, in supplement to the results of Gustafsson 1983 on social suppression of sexual maturation.

Thus, social dominance affects the marking of male bank voles in two different ways: First,subordinate males mark less frequently than dominant males; and secondly the markings of dominant and subordinate males are different in quality. Marking pheromones may not be synthesized in subordinate males (Brinck, Hoffmeyer & Westlin 1983). A substantial change may occur only after some time of interaction with females in the absence of the dominant male (Exp. 3). This change is probably correlated with increases in plasma testosterone levels which in male mice were found to result from female stimulation (Macrides, Bartke & Dalterio 1975), and which also may activate the synthesis of marking pheromones (Brinck et al. op.cit.).

BIOLOGICAL ASPECTS

Advantages of "small spot" urinations. There are several advantages for a male that marks with "small spot" urinations. First, urine is saved for marking purposes. This is important for dominant males, which have larger ranges (Brown 1966), and a higher marking activity. Secondly, the odorous effects of markings are enhanced

since the deposition of urine in small quantities is connected with deposition of preputial gland secretion. Furthermore, the distribution of odorous substance in many small concentrated spots over an area appears to affect bank voles more than does the same amount of material presented in one large spot (own observations). It is possible that trace marking is an adaptation to a specific type of arboreal environment (see Schilling 1980).

Significance of markings. I supposed that the two main advantages of male urinary marking are 1) in reducing the cost of overt aggression (male - male competition) and 2) in attracting or stimulating females (female mate choice).

The urine marking of males plays a role in social dominance/"territoriality". Dominant and subordinate male bank voles showed opposite reactions to the compound of preputial gland secretion, hexadecyl acetate, found in trace-urine, when this acetate was provided in concentrations corresponding to great amounts of trace marking of a dominant male. The dominant males reacted by significantly more approach to the acetate than to controls, whereas the subordinate males did the reverse,(p.111,114).

Also subordinate males or post-weaning subadults avoided traps carrying the smell of hexadecyl acetate. "Territorial behavior" develops in overwintered bank voles during the spring maturation (Wiger 1982), and there is a delay of several weeks between sexual maturation and the period of high trace marking activity (Christiansen 1980). This delay may well correspond to the time it takes to find a suitable "territory" and for maturation of the biosynthesis of this marking pheromone, which in males is androgen-dependent (Brinck, Hoffmeyer & Westlin 1983).

The urine marking of males also plays a role in mating. Female bank voles were more attracted by the urine odors of dominant males than by those of subordinate males (Hoffmeyer 1982), and they were also more attracted by marking urine, i.e. urine containing secretions normally added during urinary marking (Brinck & Hoffmeyer 1983). However, the pheromone, important in male--female interaction, apparently is not the hexadecyl acetate found in preputial gland secretion. Female bank voles did not respond differently, as the males did, between hexadecyl acetate and controls(p111,114). Other androgen-dependent urinary substances/pheromones of the kind which in mice have been found to stimulate sexual maturation of females and accelerate estrus-cycles (Lom-

bardii & Vandenbergh 1977) may be responsible for the attractant effects of the urine of male bank voles on females.

The results of Experiment 4 support the suggestion that marking contributes to a male bank vole's ability to mate with females; a high marking frequency in males is correlated with high mating success. Tactile cues, however, may be of major importance, especially with primiparous females (Westlin 1983).

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Responses of Female Bank Voles (*Clethrionomys glareolus*) to Dominant vs Subordinate Conspecific Males and to Urine Odors from Dominant vs Subordinate Males

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Dominant and subordinate male bank voles from stable hierarchies were used as choice objects and urine donors in tests with estrogen-treated females. In a two-choice male preference test 12 out of 15 females reliably showed a preference. All 12 preferred the dominant male. In a two-choice odor preference test the females were presented with equal quantities of normal elimination urine from dominant and subordinate males. Thirteen of fifteen females tested individually showed a preference: Twelve of them preferred the urine of a dominant, over that of a subordinate male. Other females, tested in groups of five, were presented only with the lipid (dichloromethane) fraction of urine. In this case 11 of 12 groups preferred urine lipids of dominant males to urine lipids of subordinates. Cumulative investigation times and frequency of visits were used to determine preferences. The results are discussed with regard to possible causes and biological function. Female preference here represents a case of sexual selection partly by olfaction, and may augment the reproductive success of dominant males.

Male bank voles reportedly form stable dominance hierarchies in both the field (Viitala, 1977, with *Clethrionomys rufocanus*) and the laboratory (Sørensen, 1981, with *C. glareolus*). Dominance hierarchies assign priority to a limiting resource such as food, space, and mates. Several investigations have reported the relationship between male dominance and male reproductive success in small rodents (DeFries & McClearn, 1970; Horn, 1974). The aim of the present investigations is to elucidate the role of the female bank vole in mate selection. The hypothesis was that female preference may represent a case of sexual selection partly by olfaction. It was therefore necessary to determine if female bank voles can distinguish between dominant and subordinate males, or their odors, and if they prefer one type of male or odor to the other.

Sex attractants are found in the urine of male laboratory mice (Scott & Pfaff, 1970). Furthermore, the dominance status of males may be expressed through odors in the urine (Jones & Nowell, 1974). Data on the urinary marking of male bank voles, *Clethrionomys glareolus* (Chris-

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tiansen, 1980; Hoffmeyer in prep.), indicate similar functions in this species. Christiansen (1976, 1980) has suggested that the sex attractant of male bank voles is produced by the preputial glands and mixed with the urine, as in mice (Bronson, 1966; Bronson & Caroom, 1971) and rats (*Rattus norvegicus*) (Gawienowski, 1977). Gustafsson, Andersson, & Meurling (1980) found that dominant male bank voles have larger preputial glands than subordinates, suggesting a relationship between social dominance and the rate of production of preputial gland secretion. Here two kinds of experiments were performed with female bank voles. The first tested their preference between dominant and subordinate males. The second tested their preference between urine odors from these two types of male.

MATERIALS AND METHODS

Animals

All test animals were laboratory-raised descendants from a wild stock. They were kept in standard laboratory mouse cages (40 × 20 × 15 cm); photoperiod of 18 hr light and 6 hr dark; temperature of 22 ± 5°C. Voles were sexed at 10 days of age and weaned at 18–21 days of age. Both mother and father were present until the age of weaning. At weaning, the young were separated by gender and placed in groups of five. All were housed in the same room.

Fourteen days before the tests, the females were transferred to larger cages (80 × 40 × 15 cm) similar to the normal breeding cages; they were fur-clipped for individual identification. Three days before the tests, they were weighed and treated with estradiol benzoate (Ovex B, 20 μg/kg bw) to make them receptive. The females had no sexual experience (but had been living with their parents and brothers until the age of 18 days). Nothing was done outside the test situation to determine the sexual receptivity of the females. Vaginal smears from female bank voles do not provide easily identifiable criteria as they do in the laboratory rat (Gustafsson & Westlin, personal communication). Estrous female voles are recognizable because they always seek and stay close to the male in a way clearly different from that shown during agonistic interaction.

The males used for female choice and as urine donors were from all-male groups of 3–5 individuals that had established stable dominance hierarchies. Each individual was identified as dominant or subordinate based on data gathered over at least 1 month prior to tests following the procedure of Sørensen (1981). This was done to assure that long-term physiological correlates of dominance and subordinance status with odors had developed (Bronson & Eleftheriou, 1964; Lombardi & Vandenbergh, 1977). Males used in female choice tests were always derived from the same male group to minimize agonistic interaction (by scents or sounds) between the males during the test. Ten dominant:subordinate pairs of males were available from ten all-male groups.

Urine Collection/Stimulus Odors

The urine of dominant and subordinate males was collected in special "metabolism cages" similar to the one described by Jones, Dilks, & Nowell (1973), but modified for voles with separate sleeping, feeding, and urination sections. Single males were housed in a metabolism cage overnight (from 1700 to 900) for urine collection. Each night two males, the dominant and one of the lowest ranking males, from a group were used as donors. They were then put back into the male group from which they came. Urine was never collected from a male for more than one night at a time, and at least 4 days passed before the male donor was re-used if at all. Urine was placed in separate tightly sealed vials and frozen. For Experiment 2a urine was used the day after collection. For Experiment 2b extracts from the lipid portion of urine were prepared by shaking the urine with twice its volume of dichloromethane and centrifuging for 5 min. When the layers separated the dichloromethane layer was collected and stored in tightly sealed vials in the freezer. Samples of the dichloromethane fraction were sometimes briefly thawed and refrozen. The two samples used in a test were from the same date of collection.

Procedure

The females were tested individually in their home cage. The other females in the group were removed 30 min before the start of the test and provisionally placed in a separate cage. Most of the wood shavings were also removed. Tests were run during periods of daily activity in light. Frequency of visits or cumulative investigation time were used to determine the preference of the females.

EXPERIMENT 1

Female Choice between Dominant and Subordinate Males

The two males used in each preference test with a given female, were placed in separate live-traps (UGGLAN Special with netting boxes; mesh width = 3 mm) on a Plexiglas plate adapted in size to the bottom of the female's cage. This plate was covered with filter paper fixed with tape. An 8-cm large zone ("trap zone") was delineated around each trap. The traps were attached at adjacent corners of the plate such that when placed in the female's cage one of the trap's long sides pressed against the side wall of the cage, and the entrance side against the end wall. The entrance holes of the traps were blocked. The males were put into the traps 10 min before the start of a test. While the female was briefly removed, the plate with the two traps attached was placed inside her cage. The female was then put at the middle of the wall opposite the side with the two traps. Recording started when the female had visited both traps once, and was continued for 10 min. The following criteria were used during recording: (1) "approach to" was transgression of the

trap-zone border; (2) "visits to" were stays of shorter or longer duration within the trap-zone border; and (3) "to be near" was being within the trap-zone border and intermittently sniffing the trap.

Results

All the females that approached the males (12 of 15) spent more time near the dominant male (Fig. 1, left, $p < .01$, Wilcoxon matched-pairs test, two tailed). All of these 12 females also made a greater number of visits to the dominant male than to the subordinate one (Fig. 1, right, $p < .01$, Wilcoxon, matched-pairs test, two tailed).

The females sometimes showed long latencies before they approached one of the males, but once they made a sniffing contact there was a change in the behavior.

Eight of the twelve females showed the clearest behavioral signs of being receptive: they crouched close to the trap with the selected male and performed a characteristic "flat run" alongside the trap, sometimes rubbing the flank or belly against the trap. A "flat run" is a swift running bout during which the female's body appears somehow flattened out through a lowering of the body and slight depression of the back. There may be elements of intention to lordosis in this behavior. Three of the twelve females showed lordosis in front of the trap with the male chosen.

There was no variation in general outcome with the different pairs of males used. However, one of the dominant males, known to be particularly aggressive in male–male interactions, induced flight reactions in one of the females.

Only two females urinated on the filter paper between the males, and the urinations were in a few large spots.

EXPERIMENT 2

Female Choice between Dominant and Subordinate Male Urine

(2a.) Whole urine. The same set-up as in Experiment 1 was used. The empty traps were thoroughly cleaned and covered with filter paper on top, sides and end. Immediately before the start of a test, the urine of a dominant male was placed on one trap and urine of a subordinate on the other. Equal quantities of each urine, freshly collected from the metabolism cages were distributed equidistantly in 0.02-ml drops on the filter paper covering the trap. Two drops were placed on the top, two on the long side, and one on the end side. Recording began after the female had visited each of the traps once, and the cumulated time as well as the frequency of visits were noted for 5 min.

Results. Thirteen out of the fifteen females approached urine from both dominant and subordinate males. Twelve of the thirteen females spent more time near the urine from a dominant male than near that of a subordinate male (Fig. 2, left, $p < .02$, Wilcoxon matched-pairs test,

FIG. 1. Female bank vole preference between dominant and subordinate conspecific males. Each point represents one female. (Left) Preference measured by the cumulated time spent within the trap zone of each type of male ($p < .01$). (Right) Preference measured by the frequency of visits to each type of male ($p < .01$). In one point, ◉, the values of two females coincide. (p based on Wilcoxon matched-pairs, signed-ranks tests, two tailed).

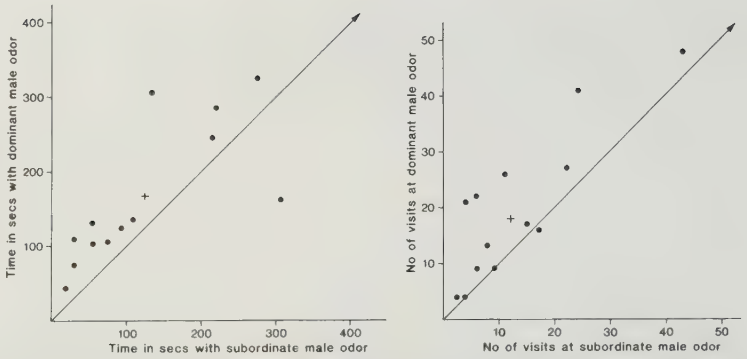

FIG. 2. Female bank vole preference between urine odors from dominant and subordinate conspecific males, in Experiment 2a, where the females were tested individually. Each point represents one female. (Left) Preference measured by the cumulated time with each type of odor ($p < .02$). (Right) Preference measured by the frequency of visits to each type of odor ($p < .01$). (p based on Wilcoxon matched-pairs, signed-ranks tests, two tailed).

two tailed). Nine of these twelve visited the trap spotted with urine from a dominant more frequently (Fig. 2, right, $p < .01$, Wilcoxon matched-pairs test, two tailed).

However, the females showed smaller differences in the cumulated times spent at the two types of odor, than was the case with the two types of male ($U = 24$, $p < .002$, Mann–Whitney U test, two tailed). Furthermore, the hesitation in choice (measured by the number of shifts between the stimulus objects) was greater in preference tests with the urine odors (Experiment 2a) than in those with the males (Experiment 1).

(2b.) Lipid fraction of urine. Here a special device for recording the response to olfactory stimuli in rodents was used (see Christiansen, Døving, & Mørk, 1977) and the stimuli were from the lipid portion (dichloromethane extract) of equal volumes of dominant and subordinate male urine. Ten microliters of each of the dichloromethane extracts was presented per test. The stimulus odors were placed inside plexiglass tubes equipped with photocells which sense the presence of the test-animal's snout. The females were tested in their home cage and in groups of five because in pilot tests we found that single females generally would show a very low frequency of approaches to the apparatus.

Twelve female groups were tested; the test period for six of these was 2 hr, and for the other six groups it was 16 hr (overnight 1700–900).

Results. All the groups approached the detecting unit with the extract of dominant male urine more often than they did the unit with extract of subordinate male urine. However, for one group tested for only 2 hr, the total number of approaches was very small. Therefore this group was omitted. For the remaining 11 groups, $p < .01$. For the groups tested overnight, $p < .05$.

DISCUSSION

(1.) Factors Determining Female Choice

There are two aspects to female choice: (a) the stimuli provided by the choice objects, and (b) the motivational state of the female.

(a) Female preference for the dominant males over the subordinates was more clear-cut than preference between the urine spots from these males. This must be due to the more composite stimulation offered by the males. Male ultrasonic vocalizations possibly occurred (Geyer, McIntosh, & Barfield, 1978; Sales & Pye, 1974) and in laboratory mice such sound emissions may show correlations with social dominance (Nyby, Dizinno, & Whitney, 1976). This is also the case with composite body odors. The ability of rats and mice to discriminate between the body odors of dominant and subordinate males (Krames, Carr & Bergman, 1969; Carr, Martorano, & Krames, 1970) is attributed to a difference in hormone secretion with higher androgen levels in the dominants and

possibly higher adrenal corticosteroid levels in subordinates (Brown, 1979). A similar situation is presumed for the bank vole.

Concerning odors in the urine of males the choice by the females was between equal quantities of urine from dominant and subordinate males collected in metabolism cages. The female's preference therefore was not due to a difference in quantity of urine as might come from the higher frequency of urine marking by dominant males (Hoffmeyer in preparation); but the preference indicates differences between the odors of urine per se from dominant and subordinate males. The results correspond to findings in laboratory mice (Jones & Nowell, 1974) and brown lemmings (Huck, Banks, & Want, 1981). Also male bank voles react differently to the two types of urine (Hoffmeyer in preparation).

The difference in odors of the urine from dominant and subordinate males may result from several things. Dominant males generally have higher testosterone levels than subordinate males. This may affect their urine odors directly and indirectly through a greater concentration of androgen derivatives or exudates in the urine from a higher metabolic rate (Stoddart, 1980), or by acting on scent glands, the secretions of which are added to the urine, possibly already from glandular tissue in the kidneys. The urinary factors may be of the kind, which advance the onset of female puberty in mice since urine from dominant male mice was found to be more effective in stimulating female maturation than was urine from subordinate males (Lombardi & Vandenbergh, 1977).

Bronson (1976) has suggested that in mice secretions from the preputial glands are added to the urine. If the secretions are added continually to all urine eliminated by a male, a higher rate of production by the preputial glands of dominant males (indicated by the larger size of these glands, p) should lead to higher concentrations of glandular secretion in their metabolic urine, as there seems to be no difference in the quantities of urine excreted by dominant and subordinate males (Desjardins, Maruniak, & Bronson, 1973, *Mus musculus;* Hoffmeyer unpublished, *Clethrionomys glareolus*).

Glandular secretions could be added to the urine specifically during urine marking. Christiansen (1980) has suggested that in the bank vole preputial secretions are deposited by males in the special "trace-urinations", which according to my own results are much more frequent in dominant than in subordinate male bank voles. The papers by Christiansen suggest a dual function of the preputial glands. Besides the hypothesis of a sex attractant function the scent of preputial secretions may signal aggression/teritoriality/social dominance, as originally suggested by Brown and Williams (1972) in their monograph. This could also explain the interest that females show in preputial odors of their own sex.

Whatever the cause, the stronger effects of the urine per se from dominant males found here with voided metabolic urine will be enhanced by the higher urinary marking frequency of these animals. If, furthermore,

some specific attractant is added during marking only, and perhaps just with special types of urinations, female interest should become more pronounced by using extracts of marking urinations from dominant males instead of the urine collected in metabolism cages or bladder urine. Behavioral tests and chemical analyses are in process concerning this question.

(b) Factors influencing female preferences are the hormonal state and experience of the females (e.g., Doty, 1972; Nevo, Bodmer, & Heth, 1976; Caroom & Bronson, 1971; Hayashi, 1979).

In the present experiments, all the female bank voles were estrogen treated. The hormonal control of female preference is unclear (Brown, 1979) but naive females have to be receptive before they show a preference for male urine odor (Carr, Loeb, & Dissinger, 1965, for rats; Jones & Nowell, 1974, for mice), and estrogen may enhance the interest in odor from male preputial glands in ovariectomized female mice (Bronson, 1976).

The female bank voles had no sexual experience, but had been living with their mother, father, and brothers until the age of weaning. Sexual experience does not seem to be necessary for female mice or rats to show preferences for the urine odors of sexually active males over those of castrated males (review by Brown, 1979), but it enhances their preference for odor from male preputial glands (Caroom & Bronson, 1971). However, also, juvenile experience with the mother's odors may determine female preference for preputial odors (Hayashi, 1979). It did not seem to matter that the female bank voles were not familiar with the males used.

(2.) Possible Biological Functions

Females may play an active role during mate seeking (Mazdzer, Capone, & Drickamer, 1976) and possibly also with mate choice in polygynous mating systems (Orians, 1969; Kleiman, 1977).

Female preference for a dominant male may increase her chances of successful reproduction in several respects. Obviously, a female might (perceptually) combine social dominance and sexual attraction signals, as they are possibly two aspects of the trait. In natural populations a mechanism of female choice may be particularly important for dispersing females. It assists to increase the contribution of dominant males to the gene pool. In consideration of the fact that the dominant males used in the present experiments had all been identified through their aggressiveness to strange male intruders, studies concerning the heritability of aggression in this small rodent species are suggested for future research.

Sexual selection in visually oriented animals like birds results in differences between males in coloration and structures used in displays. Many mammals for which olfaction is important may depend on differences in male odor (Blaustein, 1981). This is suggested by differences in scent-gland size between males found in many mammal species (Mykytowycz

& Dudzinski, 1966; Bronson & Marsden, 1973; Gustafsson et al., 1980). Tests on female preference between the odors from males of different social status (Jones & Nowell, 1974; Huck et al., 1981; and the present bank vole results) provide some evidence of the role of male odor in intersexual selection.

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MARKING URINE AND PREPUTIAL GLAND SECRETION OF MALE BANK VOLES (CLETHRIONOMYS GLAREOLUS L.); CHEMICAL ANALYSES AND BEHAVIOURAL TESTS

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ABSTRACT

Urine and preputial gland secretion of male bank voles were analyzed by gas chromatography (GC) and mass spectrometry (MS) (selected ion monitoring, SIM). GC/MS analyses showed the presence of hexadecyl acetate in preputial gland secretion and in marking urine but not in metabolic urine. Female bank voles responded more strongly to marking urine of males than to metabolic urine and they responded more to preputial gland secretion (pure or added to urine) than to metabolic urine. Dominant males spent more time and marked more frequently in response to hexadecyl acetate-enriched urine than to urine alone. The opposite reaction was shown by subordinate males. The results suggest that the acetate functions in bank voles' dominance interactions.

INTRODUCTION

Urine marking behaviour in bank voles and the development of their preputial glands are correlated with sex and sexual maturation (Johnson 1975, Christiansen, Wiger and Eilertsen 1978, Christiansen 1980). Male Clethrionomys voles form stable dominance hierarchies observed both in the field (in Clethrionomys rufocanus, Viitala 1977) and in the laboratory (in Clethrionomys glareolus, Sörensen 1981). In mice (Mus musculus) males demonstrate their dominance through higher urinary marking frequencies (Desjardins, Maruniak and Bronson 1973) and through qualitative differences in their urine (Jones and Nowell 1974).

In the bank vole, dominant males have larger preputial glands than subordinates (Gustafsson, Andersson and Meurling 1980). This suggests the possibility of a correlation between social dominance and production of preputial gland secretion. Also dominant male bank voles mark significantly more frequently than subordinates when confronted with different types of intruders (Hoffmeyer in 1983). Female bank voles are able to distinguish urine odours of dominant and subordinate males and are more attracted by those of the dominants (Hoffmeyer 1982). Christiansen (1976, 1980) suggested that a sex attractant of male bank voles is produced by the preputial glands and mixed with the urine as in mice (Bronson 1966, 1976).

The aim of this paper was to relate the behavioural reactions of male and female bank voles to the chemical composition of urine and preputial secretion of males.

MATERIALS AND METHODS

ANIMALS

The bank voles (Clethrionomys glareolus) were all descendants of a wild stock kept in the laboratory (Gustafsson et al. 1980). They were kept in plastic mouse cages (40x20x15 cm) on a photoperiod of 18 hrs light and 6 hrs dark, and at a temperature of 22 ± 5° C. They were sexed at the age of 10 days and weaned at 18-21 days, when they were caged in groups of five, males and females separate. All individuals were housed in the same room, which presumably stimulated their sexual maturation. The male urine donors as well as the test animals were selected from all--male groups of 3-5 individuals with stable dominance hierarchies. To determine social dominance in groups of bank voles, we used the intruder method modified after Sörensen (1981). According to weekly testing, the males had held the same social status for at least one month prior to the chemical testing. This was to assure long-term physiological correlates of social status, which may affect male odours (review by Brown 1979).

COLLECTING URINE AND PREPUTIAL GLAND SECRETION

Marking urine for behavioural tests was collected on 265x130 mm filter paper, pre-washed in methylene chloride. Urinations from 10 min of marking by individual dominant males (average total trace length 100 cm) delineated under UV-light at 254 nm, were cut, and the paper-pieces extracted with methylene chloride (5 ml). The extracts were stored at -20° C. In collection of markings for chemical analyses the test animal was allowed to mark for 10 min on a glass plate (200x200 mm). The deposited material was extracted with a total amount of 0.5 ml methylene chloride. The solution was concentrated to 10 µl and stored at -20° C until examination.

Metabolic urine was collected in cages described by Jones, Dilks and Nowell (1973), but modified for voles to have separate sleeping, feeding and urination sections. The males were kept individually in the cages overnight. A male donor was used for urine collection at intervals of at least 4 days. The urine was extracted with twice its volume of methylene chloride. The organic fraction was stored at -20° C. Metabolic urine that was to be compared with marking urine was dropped on filter paper, and then extracted similarly. For the behavioural tests 5 µl were used as odour sample. For chemical analyses, organic fractions from 8 ml urine were concentrated almost to dryness.

Preputial gland secretion from killed specimens was squeezed from the large efferent ducts and transferred to glass capillaries together with a small amount (40 µl) of methylene chloride per specimen. For behavioural tests 5 µl from one specimen was used after evaporation of the solvent. For chemical analyses pooled samples from 4 specimens were used. The solution was concentrated to 10 µl of which 1 µl samples were used to obtain mass spectra.

GAS CHROMATOGRAPHY (GC)

GC was performed on a Perkin Elmer model 900 equipped with a flame ionization detector (FID). A 25 ml fused silica capillary column (i.d. 0.20 mm) dynamically coated with OV-101 was used. The injector temperature was 230° C. The split valve was opened 60 s after injection. After another 120 s the column temperature was linearly increased by 4° C min^{-1} from ambient to 220° C.

MASS SPECTROMETRY (MS)

A Ribermag R10-10c quadrupole GC/MS data acquisition system equipped with a Carlo Erba Model 4160 GC was used. Separation was performed on a 25 m fused silica column, i.d. 0.20 mm, dynamically coated with SE-54 as stationary phase. One µl samples were injected splitless at a column temperature of 100° C. The split valve was opened 30 s after the injection and the temperature increased linearly by 10° min^{-1} after 2 min to 220° C, where it was held constant. The injector temperature was 230° C. The electron energy used was 70 eV in both electron impact ionization (EI) and in chemical ionization (CI). The temperatures in the ion source were 125° C and 100° C, respectively. Ammonia at 1 torr in the ion source served as reactant gas.

TESTINC BANK VOLE RESPONSES

Females. Females were tested between 2-3 months of age. Fourteen days before testing they were transferred to larger cages (80 x 35 x 20 cm), and were fur-clipped for individual identification. They were weighed and treated with extradiol benzoate (OVEX B, 20 µg/kg bw), once per day, for 3 days before the test. Microtines are reflex ovulators. They do not like mice or rats (spontaneous ovulators) come into estrous regularly. The estrogen-treatment was given to facilitate estrous, which enhances female responsiveness

to male stimuli. Two hours before testing an adult male was placed in their cage in a small wirenet box. It was left there for one hour while frequent observations were made to assess the females' estrous state. This and the previous presence of father and brothers constituted the females' only experience of males. Estrous female voles are recognizable because they always seek and stay close to the male in a way clearly different from that shown during agonistic interaction. Vaginal smears from female bank voles do not provide easily identifiable criteria as they do in the laboratory rat (Gustafsson and Westlin pers.comm.).

Exp.1. A special device for bioassay of response to olfactory stimuli in rodents was used (described by Christiansen, Döving and Mörk 1977). It consists of a 4-chamber choice box of plexiglass/aluminium abt. 20 x 10 x 5 cm. Four plexiglass tubes, with an inner diameter of 20 mm, open through holes in the longside. Each is provided with infrared photocells. Smaller tubes with an outer diameter of 19.5 mm can be inserted into the fixed tubes. The odour to be tested is applied on a filter paper strip mounted on a cork which is placed in the smaller tube. The device is simple to clean and operates automatically. It was placed in the females' home cage. In each test, 3 samples were presented (2 odour samples + 1 blank) in randomized positions. The females were tested groupwise (with only familiar group mates) for two hours. They could approach the apparatus freely and sniff the odour samples. Only the cumulated scores per stimulus per test period were calculated.

Exp.3. (for females see p104).

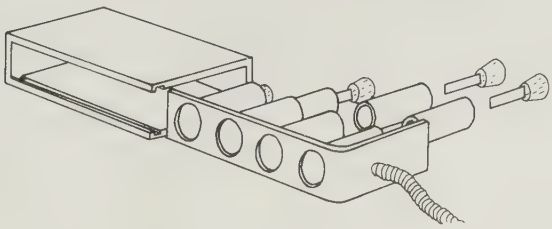

Fig. 1. Olfactometer used in Exp. 1 with females. Designed by Christiansen, Döving & Mörk, 1977.

Males. The males were between 3-6 months of age. A choice situation was used, where the animals' urine marking responses could be recorded.

Exp.2. The males were presented with two samples per test. These were presented in separate compartments (of plexiglass 20 x 9 x 12 cm) lined with filter paper on the floor. These compartments were connected to the test male´s home cage by short (6 cm long) removable plexiglass tubes (Ø 4 cm). The males were tested individually. They were isolated in their home cage, for 1 hour before testing. Immediately before the start of a test, each odour sample was placed at the far end of one compartment, on the floor, and the compartments were connected to the male's cage. A test started when the test animal first time entered a compartment and was continued for 10 minutes. The urine marking frequency of each male was determined as described in Hoffmeyer 1983a by counting number of marks present on each sheet of filter paper. The difference in number of marks deposited in the two compartments during the test period was calculated for each male (Tab. 2).

Exp.3. In the last experiment (see Tab. 3) testing male and female responses to hexadecyl acetate, the same set-up was used, but only the cumulated times spent in each compartment for 5 min active time were compared.

In all 3 experiments each animal was presented with only one stimulus-combination per day. On the next day a different combination was given, using samples from a new donor male. The animals were not tested with more than two combinations, which were given in random order. After the presentation of a combination to an animal, the scores per stimulus were compared and the difference calculated. At the end of a test series, a Wilcoxon matched-pairs signed-ranks test was applied. The females tested with the olfactometer (Exp.1) could freely approach the apparatus and choose between the stimuli. Testing in groups proved to be necessary to overcome the timidity of the females. The drawback was that the statistical unit then was the group. In the other type of test situation used in Exp. 2 and Exp. 3, the test animal always had its home cage as an alternative to the stimulus compartments, so that choices could proceed independently.

Chemical analyses. Gas chromatographic analyses of preputial gland secretions indicated only small amounts of material of high volatility (Fig. 2a and b). GC retention times of peaks (1) and (2) coincided with those of synthetic hexadecyl and octadecyl acetate, respectively. Mass spectrometric examination of the volatile material corresponding to peaks (1) and (2) revealed that the fractions contained only minute amounts of acetate. Fig. 3 shows the mass spectrum of a compound isolated from pooled extracts of preputial gland secretion of 4 dominant males. The fragmentation pattern was identical with that of authentic hexadecyl acetate. The peaks at m/z 61 and m/z 116 are characteristic for saturated acetates (Budzikiewicz et al. 1967). No molecular ions can be seen and the ions of highest mass (at m/z 224) are formed by the loss of acetic acid (M-60) from the molecule. Although we looked intensively for octadecyl and octadecenyl acetates, we could not find any traces. Fig. 4 shows a mass fragmentogram of preputial gland secretion from a dominant male, in the region of the GC-retention time for hexadecyl acetate, with the mass spectrometer arranged for selected ion monitoring (SIM). Chromatogram (a) represents the integrated signals of ions of m/z 61.0 and chromatogram (b) the signals of ions of m/z 224.2 = M-60, strongly suggesting the presence of hexadecyl acetate in the preputial gland secretion. Also a corresponding fragmentogram of extracts of marking urine indicates the presence of this acetate in the urine (Fig. 5). The same mass fragmentographic analysis was performed on an extract of metabolic urine from a dominant male. Although a very large volume (8 ml) of urine was extracted, no hexadecyl acetate was detected (Fig. 6 above). Fig. 6 below shows the same extract with 600 pg of authentic acetate added. Interestingly, the fragmentogram (Fig. 6 above) indicated the presence of ions of m/z 224.2 formed by cleavage of a compound with a somewhat shorter GC retention time than that of hexadecyl acetate. Whether these ions reflect the presence of a hexadecyl acetate isomer in the metabolic urine or originate from an entirely different compound is not known.

Reactions of bank voles. Female bank voles were more attracted to marking urine than to metabolic urine (Table 1). The females also reacted significantly more frequently to urine supplemented with preputial gland secretion and to pure preputial gland secretion than to metabolic urine. Additional tests were made with

dominant males (Table 2). They marked more frequently as a reaction to a mixture of urine and preputial gland secretion than to metabolic urine.

Dominant males in general spent more time with acetate-enriched urine than with controls (Table 3). They also performed urine marking more frequently in areas impregnated with acetate-enriched urine. Conversely the subordinate males spent less time with acetate-enriched urine (Table 3). In females the behaviour was less consistent. The rank of the females was not known and female bank voles generally do not form as distinct dominance hierarchies as males do. The three females reacting positively behaved aggressively within their group, in contrast to the other females.

DISCUSSION

Whether preputial gland secretion is released in the urine or not has been a matter of debate. It has been uncertain whether the secretion is released only in connection with urinary marking or in all eliminated urine. According to Bronson and Caroom (1971), a lipophilic factor, strongly attractive to sexually experienced female mice was found in a preputial gland homogenate and externally voided urine, but not in bladder urine or urine from preputial-ectomized males. Bronson (1976) concluded that preputial secretions probably are mixed with urine during marking. Similar suggestions were made about the bank voles by Christiansen (1980).

The results of this study show that preputial secretion is connected with urinary marking. First, hexadecyl acetate is present in the preputial gland secretion and is deposited in marking urine, but not in metabolic urine. Second, both males and females react strongly to urine mixed with preputial gland secretion when this mixture is presented as an alternative to metabolic urine.

Only dominant individuals reacted positively to the hexadecyl acetate. This suggests that the acetate has a function in dominance/territorial interactions. Furthermore, dominant males mark more frequently than subordinates (Hoffmeyer 1983) and have larger preputial glands (Gustafsson et al. 1980).

Male bank voles generally reacted clearly to the hexadecyl acetate, while this was not the case with the females. This indicates that the acetate is more important in male - male competition than in intersexual interactions. The lack of a clear ten-

dency in the results with the females was unexpected, because the females were attracted by marking urine and by preputial secretion added to urine. However, females may need the addition of more specific cues.

Testosterone is necessary for urinary marking in male mice (Maruniak, Desjardins and Bronson 1977, Bronson 1979). The same appeared to be the case in bank voles (Christiansen 1980). The synthesis of the marking compound hexadecyl acetate was found to be androgen dependent; testosterone treatment of subordinate male bank voles caused a change in the histology of their preputial glands and an increase of the amount of hexadecyl acetate (Brinck, Hoffmeyer and Westlin 1983).

Social dominance relations may also affect the external release. In mice, urinary marking was inhibited in subordinate males, even when their testosterone levels were artificially elevated (Maruniak, Desjardins & Bronson, op. cit.).

In the bank vole high marking frequency was correlated with high social status, and dominant male bank voles reacted to hexadecyl acetate (Tab. 3 and Hoffmeyer in prep.). Inhibited release of this compound in subordinate male bank voles could be one way of signalling subordination. In nature such low status signalling may be advantageous if subordinate males stay within the range of dominants (review by Rohwer and Ewald 1981).

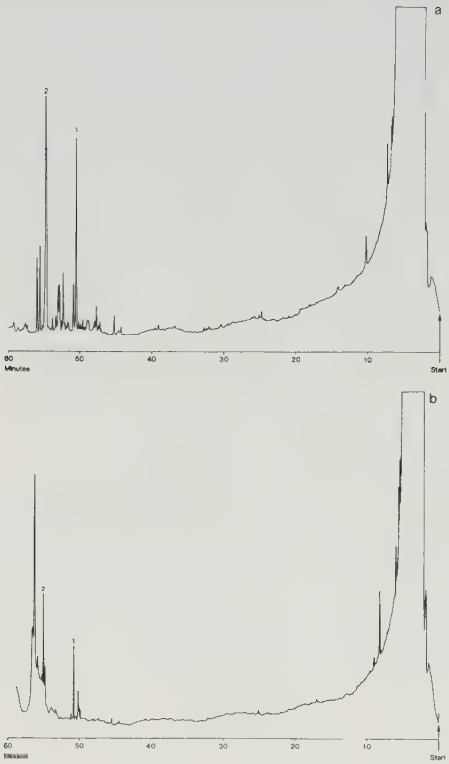

Fig. 2. Gas chromatograms of components in the preputial gland secretion from a) a dominant male bank vole and b) a subordinate male bank vole.

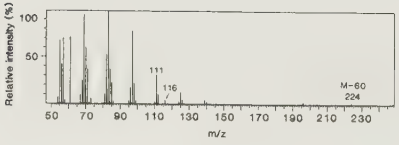

Fig. 3. Mass spectrum of a component isolated from a methylene chloride extract of preputial gland secretion of a dominant male bank vole.

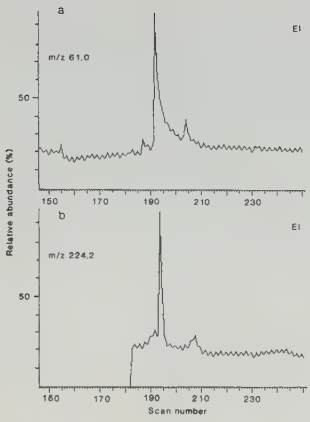

Fig. 4. Mass fragmentograms (SIM) of an extract of preputial gland secretion of a dominant male bank vole, monitoring of m/z 61.0 and m/z 224.2.

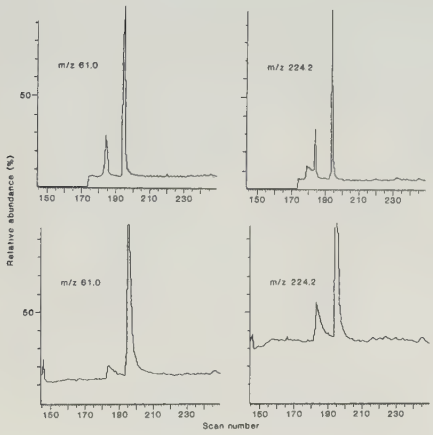

Fig. 5. Mass fragmentograms of a lipid extract of marking urine from one dominant male bank vole (above) and the extract with 600 pg n-hexadecyl acetate added (below). Monitoring as in Fig. 4.

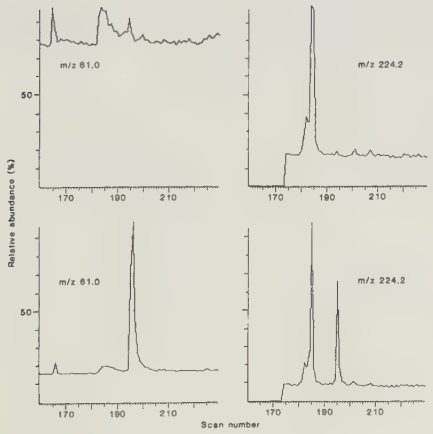

Fig. 6. Mass fragmentograms monitoring as in Fig. 4 of a lipid extract of metabolic urine from one dominant male (above) and same extract with 600 pg n-hexadecyl acetate added (below).

TABLE 1 (Exp.1)

Responses of female bank voles to marking and metabolic urine of conspecific dominant males. The females were tested by an olfactometer (Christiansen, Döving and Mörk 1977) in groups of five, (p 103).

N	Approach Frequency (mean ± SE)		P (two-tailed)
	Marking urine	Metabolic urine	
12	21.3 ± 3.0 (63%)	12.4 ± 2.3 (37%)	< 0.01 (T = 0)
	Urine mixed with Preputial Secretion	Metabolic urine	
12	12.9 ± 1.9 (61%)	8.4 ± 1.8 (39%)	0.01 (T = 3)
	Preputial Secretion	Metabolic urine	
12	12.7 ± 2.3 (60%)	8.6 ± 2.2 (40%)	< 0.05 (T = 12.5)

N = number of female groups
T = critical value in the Wilcoxon Matched-Pairs Signed-Ranks Test (Siegel 1956).

TABLE 2 (Exp.2)

Urinary marking frequency of dominant male bank voles exposed to 5 µl methylene chloride extracts of urine mixed with preputial secretion or metabolic urine of conspecific males. The samples were provided in separate compartments connected to the home cage. Males tested individually.

N	Urinary Marking Frequency (mean ± SE)		P (two-tailed)
	Urine mixed with Preputial Secretion	Metabolic urine	
12	39.4 ± 5.7 (60%)	26.6 ± 3.7 (40%)	< 0.01 (T = 5)

N = number of individuals tested
T = critical value in the Wilcoxon Matched-Pairs Signed-Ranks Test (Siegel 1956).

TABLE 3 (Exp. 3)

Bank vole responses to hexadecyl acetate (1 ng). Acetate and control samples were provided in separate compartments connected to the home cage of the test animal. Test period 5 min active time. The animals were tested individually.

	N	Number of animals spending most time in compartment containing urine with acetate	urine without acetate	P^a	Cumulated time in secs. spent in compartment containing urine with acetate (mean secs±SE)	urine without acetate (mean secs±SE)	P^b
Dominant males	12	11	1	<0.001	87.4 (±16.2)	58.5 (±11.7)	0.05
Subordinate males	10	0	10		57.1 (±11.8)	67.9 (±13.5)	<0.01
Females	12	3	9		68.4 (±13.8)	82.8 (±14.1)	NS

N = number of individuals tested

Statistical methods; Dominant males ≠ subordinate males. P^a: X^2-test. Difference in cumulated times recorded for each individual P^b: Wilcoxon matched-pairs signed ranks test (two-tailed).

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APPENDIX TO VI

ADDITIONAL EXPERIMENT ON RESPONSES OF BANK VOLES TO THE MARKING COMPOUND HEXADECYL ACETATE

The choices of different types of animals between live traps (UGGLAN SPECIAL) with hexadecyl acetate at the entrance hole vs traps with solvent heptane (controls) were studied. Paper-tape (Ruban Adhesif Scotch) was fixed on the base of the entrance hole, and provided with stimulus samples In each test, half of the traps were provided with 1 µl heptane solution of hexadecyl acetate (~ 1 ng) and the other half with 1 µl pure heptane (controls). Traps with hexadecyl acetate and control traps were presented pairwise with random site positions. The tape was removed, the traps machine-washed with boiling water and strong detergent for 12 minutes, and air dried after each trapping test.

The results are presented in Tabs. 4-7. In males, social status and sexual maturation determined the response to hexadecyl acetate (Tabs. 4-6), while no clear tendency could be found in the responses of females (Table 7). Dominant males were generally attracted by the traps with hexadecyl acetate, while subordinate males or subadults seemed to avoid them. The young males tested just after weaning (age 18 days) mostly avoided the traps with the acetate, while it was just the opposite 14 days later about the start of sexual maturation of these males (Tab. 6).

The results are similar to those of VI (Tab. 3) and support the conclusion that the marking compound has a function in male-male dominance interactions. A dominant male should indeed approach a potential competitor, while subordinates should keep away as their costs of interaction would be too high. There are two possible causes of the differences found with the young males (Tab. 6): Hormones and learning (Brown 1979). Androgens may determine responsiveness to specific compounds and experience of subordination may determine aversiveness. I suggest that 1) the newly weaned a) were not yet in a hormonal condition to respond and/or b) had the recent experience of social subordination to their father; 2) the results with the older young could be due to a) increasing androgen levels in these males and b) to the waning of the effects of negative conditioning to the father, their own dominance relations being not yet established.

Table 4 . Trapping dominant and subordinate male bank voles in live traps (UGGLAN Special) with hexadecyl acetate, vs solvent heptane (control) on entrance hole. Acetate- and control traps presented pairwise. Males tested in groups of 4 (1 dominant + 3 subordinates) over two consecutive nights (= 1), and 2). 6 male groups tested. The figures represent all animals trapped; but statistical testing (Fisher test) based on groups for 1st trapping night.

Test animals	N	trapping night	number trapped in trap with hexadecyl acetate	number trapped in trap with heptane (control)	P
A:Dominant males	6 (6x1)	1)	5	1	0.025
		2)	5	1	
B:Subordinate males	18 (6x3)	1)	0	18	
		2)	5	13	

Table 5 . Trapping dominant and subordinate male bank voles in live traps (UGGLAN Special) with hexadecyl acetate, vs solvent heptane (control) on entrance hole. Acetate- and control traps presented pairwise. Males (different from those of Tab.4) were tested individually; each male once.

Test animals	N	number trapped in trap with hexadecyl acetate	number trapped in trap with heptane (control)	P
A:Dominant males	10	9	1	0.005^{a}
B:Subordinate males	10	1	9	0.01^{b}
C:Young subadult males (at weaning)	10	3	7	

P = level of significance according to Fisher test: ^{a} for A compared with B; b for A compared with C.

Table 6. Trapping young male bank voles in traps (UGGLAN Special) with hexadecyl acetate vs solvent heptane on the entrance hole. Acetate and control traps presented pairwise. The males were tested individually at two different ages: 1)at weaning (age 18 days), 2) 14 days after weaning (age 32 days).

		N	number trapped in trap with hexadecyl acetate	heptane (control)
Young males	1)	26	9	17
	2)	26	16	10

Table 7. Trapping female bank voles in traps (UGGLAN Special) with hexadecyl acetate vs solvent heptane on the entrance hole. Acetate and control traps presented pairwise. The females were tested individually. The young females were tested at two different ages: 1)at weaning (age 18 days), 2) 14 days after weaning (age 32 days). The adult females were lactating.

		N	number trapped in trap with hexadecyl acetate	heptane (control)
Young females	1)	25	10	15
	2)	25	12	13
Adult females (lactating)		20	11	9

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